PB84-182369

Approaches to Risk Assessment for Multiple Chemical Exposures

(U.S.) Environmental Protection Agency
Cincinnati, OH

Mar 84

U.S. Department of Commerce
National Technical Information Service
NTIS

United States
Environmental Protection
Agency

Environmental Criteria and
Assessment Office
Cincinnati OH 45268

EPA-600/9-84-008
March 1984

PB84-182369

Research and Development

EPA

Approaches to Risk Assessment for Multiple Chemical Exposures

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/9-84-008	2.	3. RECIPIENT'S ACCESSION NO. PB84 182369
4. TITLE AND SUBTITLE Approaches to Risk Assessment for Multiple Chemical Exposures		5. REPORT DATE March 1984 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Dr. Jerry F. Stara Dr. Linda S. Erdreich		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS same as Block 12		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Criteria and Assessment Office Office of Research and Development U.S. Environmental Protection Agency Cincinnati, OH 45268		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600/22

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The Environmental Criteria and Assessment Office (ECAO) in Cincinnati has developed health risk assessment methods for chronic exposure to single chemical from a single route of exposure. Risk assessments for carcinogens associated an exposure level with a particular incidence of cancer using a non-threshold model which is linear at low doses. For systemic toxicants, the no-observed-adverse-effect level (NOAEL)/Uncertainty Factor approach was used to estimate an acceptable daily intake (ADI). In order to develop methods for assessing risk from exposure to mixtures of chemicals, all relevant routes and all exposure durations must be considered. The basis of the single chemical approach is constantly updated to keep pace with developments in pharmacokinetics, species and route extrapolation, mathematical models, additivity and interaction. These and other relevant issues were addressed during a 2-day workshop on "Approaches to Risk Assessment for Multiple Chemical Exposures" held by the U.S. Environmental Protection Agency in Cincinnati, Ohio on September 29 and 30, 1982. The workshop was attended by 50 scientists from EPA and private industry. This document presents the results of this workshop, including presentations, critiques and discussion for each of the 11 subtopics covered.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 286
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/9-84-008
March 1984

APPROACHES TO RISK ASSESSMENT FOR MULTIPLE CHEMICAL EXPOSURES

Dr. Jerry F. Stara, Director, ECAO
Editor
Environmental Criteria and Assessment Office
Cincinnati, Ohio 45268

Dr. Linda S. Erdreich
Technical Editor
Environmental Criteria and Assessment Office
Cincinnati, Ohio 45268

Contract No. 68-03-3111 by Dynamac Corporation

Contract No. 68-03-3156 by ERCO/Energy Resources Co. Inc.

Environmental Criteria and Assessment Office
Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

ia

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

TABLE OF CONTENTS

Page

SYSTEMIC TOXICANTS (September 29, 1982) 1

Acceptable Daily Intake 1

Presentation 2
Critiques 23
Discussion 36
References 40

Interspecies Conversion of Dose and Duration of Exposure 43

Presentation 44
Critiques 47
Discussion 58
References 62

Risk Assessment for Less-Than-Lifetime Exposure 64

Presentation 65
Critiques 69
Discussion 72
References 74

Incidence and/or Severity of Effects 75

Presentation 76
Critiques 78
Discussion 86
References 90

Route-to-Route Extrapolation and the Pharmacokinetic Approach . . . 91

Presentation 92
Critiques 101
Discussion 114
References 116

Multiple Route Exposures 118

Presentation 119
Critiques 124
Discussion 130
References 131

TABLE OF CONTENTS (cont.)

Page

The Impact of Carcinogens in Risk Assessment of Chemical Mixtures 132

Presentations 133
Discussion. 143
References. 164

HEALTH ASSESSMENT OF EXPOSURES TO CHEMICAL MIXTURES (September 30, 1982) 172

Outline of Issues and Review of Present Approaches 173

Presentations 174
Discussion. 175
References. 182

Assessment of Exposure 183

Presentations 184
Discussion. 186

Subpopulations at Greater Risk 189

Presentation. 190
Critiques 204
Discussion. 208
References. 215

Biological Bases of Toxicant Interactions and Mathematic Models. . 219

Presentation. 220
Critiques 240
Discussion. 252
References. 258

Summation of Meeting 264

Concluding Comments. 273

WORKSHOP PARTICIPANTS

ECAO-Cin AUTHORS AND REVIEWERS

Dr. Jerry F. Stara
Toxicologist (Director)

Randall J.F. Bruins
Environmental Scientist

Dr. Michael L. Dourson
Toxicologist

Dr. Linda S. Erdreich
Epidemiologist

Dr. Richard C. Hertzberg
Biomathematician

Mr. Steven D. Lutkenhoff
Acting Deputy Director

Dr. Debdas Mukerjee
Environmental Health Scientist

Ms. Cynthia Sonich Mullin
Epidemiologist

Dr. William E. Pepelko
Toxicologist

Ms. Annette Pressley
Task Manager

EXTERNAL PEER REVIEWERS AND PARTICIPANTS

Dr. Roy Albert
Inst. of Environmental Medicine
Tuxedo, New York

Dr. Julian Andelman
University of Pittsburgh
Graduate School of Public Health
Pittsburgh, Pennsylvania

Dr. Eula Bingham
Kettering Laboratory
College of Medicine
University of Cincinnati
Cincinnati, Ohio

Dr. Daniel M. Byrd, III
Health and Safety Regulation Dept.
American Petroleum Institute
Washington, DC

Dr. Tom Clarkson
Division of Toxicology
School of Medicine and Dentistry
University of Rochester, New York

Dr. Herb Cornish
School of Public Health
University of Michigan
Ann Arbor, Michigan

Dr. Kenny S. Crump
Science Research Systems
Ruston, Louisiana

Dr. Robert Cumming
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Dr. Patrick Durkin
Syracuse Research Corporation
Syracuse, New York

Mr. Kurt Enslein
Health Designs, Inc.
Rochester, New York

Mr. William Gulledge
Chemical Manufacturers Association
Washington, DC

Dr. Rolf Hartung
School of Public Health
University of Michigan
Ann Arbor, Michigan

Dr. Donald Hughes
Procter and Gamble Company
Cincinnati, Ohio

EXTERNAL PEER REVIEWERS AND PARTICIPANTS (cont.)

Dr. Marvin Legator
University of Texas Medical School
Dept. of Preventive Medicine and Community Health
Galveston, Texas

Dr. Richard Kociba
Dow Chemical
Midland, Michigan

Dr. Myron Mehlman
Mobil Oil Company
Princeton, New Jersey

Dr. Sheldon D. Murphy
Dept. of Environmental Health
University of Washington
Seattle, Washington

Dr. Robert A. Neal
Chemical Industrial Institute of Toxicology
Research Triangle Park, North Carolina

Dr. William Nicholson
Department of Environmental Health
Mt. Sinai Hospital
New York, New York

Dr. Ian C.T. Nisbet
Clement Associates
Arlington, Virginia

Dr. Ellen O'Flaherty
Kettering Laboratory
University of Cincinnati
Cincinnati, Ohio

Dr. Magnus Piscator
University of Pittsburgh
Center of Excellence
Pittsburgh, Pennsylvania

Dr. Reva Rubenstein
National Solid Waste Management Organization
Washington, DC

Dr. Marvin Schneiderman
Clement Associates
Arlington, Virginia

Dr. Harry Skalsky
Reynolds Metals Company
Richmond, Virginia

Dr. Robert Tardiff
Life Systems/ICAIR
Arlington, Virginia

Dr. James L. Whittenberger
Occupational Health Center
University of California
Irvine, California

Dr. James Withey
Food Directorate
Bureau of Chemical Safety
Ottawa, Canada

Dr. Ronald Wyzga
Electric Power Research Institute
Palo Alto, California

OTHER GOVERNMENT SCIENTISTS

Dr. Judith Bellin
U.S. EPA
Washington, DC

Dr. James W. Falco
U.S. EPA
Washington, DC

Dr. Les Grant
Director, ECAO
U.S. EPA
Research Triangle Park, North Carolina

Dr. Arnold Kuzmack
U.S. EPA
Washington, DC

Dr. William Lappenbusch
U.S. EPA
Washington, DC

Dr. Si Duk Lee
U.S. EPA
Research Triangle Park, North Carolina

OTHER GOVERNMENT SCIENTISTS (cont.)

Dr. Robert McGaughy
U.S. EPA
Washington, DC

Dr. James Melius
DSHEFS, NIOSH
Cincinnati, Ohio

Dr. James Murphy
U.S. EPA
Washington, DC

Dr. Arthur Pallotta
U.S. EPA
Washington, DC

William B. Peirano
U.S. EPA
Cincinnati, Ohio

PREFACE

The Environmental Criteria and Assessment Office (ECAO) in Cincinnati has prepared methodologies for deriving ambient water quality criteria and for conducting risk assessments on a specific group of solvents. The methodology for deriving ambient water quality criteria focused on chronic exposure to a single chemical from a single route of exposure. The solvent methodology expanded this approach to consider the effect(s) of a single chemical by all relevant routes of exposure (oral, dermal and inhalation) for all of exposure duration (acute, short-term, subchronic and chronic). In both methodologies, risk assessments for carcinogens associated an exposure level with a particular incidence of cancer using a non-threshold model which is linear at low doses. For systemic toxicants, the no-observed-adverse-effect level (NOAEL)/Uncertainty Factor approach was used to estimate an acceptable daily intake (ADI).

The current Multichemical Health Risk Assessment methodology which ECAO is attempting to develop is intended to be used in conducting site-specific risk assessments on hazardous waste disposal facilities. In developing this methodology, it will be assumed that other offices in the Agency will be able to make reasonable estimates of daily doses from oral, dermal and inhalation routes and will be able to adequately characterize the length of exposure and population exposed. Ideally, the methodology developed by ECAO would be used to estimate from the available exposure data the types of health effects which might be expected, the incidence of these effects, as well as an estimate of the relative hazard of each facility. Thus, some of the major areas for methodologic development include a reasonable approach for multiple chemical exposures, a system for combining or weighting adverse effects, and the selection of a reasonable extrapolation model for toxic effects.

These and other relevant issues were addressed during a 2-day workshop on "Approaches to Risk Assessment for Multiple Chemical Exposures" held by the U.S. Environmental Protection Agency in Cincinnati, Ohio on September 29 and 30, 1982. The workshop was attended by 50 scientists from EPA and private industry. The first day of the workshop focused on the subject of "Systemic Toxicants". Presentations were made on seven aspects of this topic. Each presentation was followed by prepared critiques from other attendees and then a discussion session. Presentations on the second day of the workshop addressed the subject of "Health Assessment of Exposures to Chemical Mixtures".

This document presents the results of this workshop, including presentations, critiques, discussion and references for each of the 11 subtopics covered. A summary of the workshop is presented at the end of this document, as well as concluding comments submitted by participants some time after the workshop.

Dr. Jerry F. Stara
ECAO, OHEA, U.S. EPA

SYSTEMIC TOXICANTS

Acceptable Daily Intake

Presentation:	Dr. Michael Dourson ECAO, OHEA, U.S. EPA
Critique:	Dr. Thomas Clarkson University of Rochester
Critique:	Dr. Harry Skalsky Reynolds Metals Company
Critique:	Dr. Arthur Pallotta U.S. EPA, Office of Solid Waste and Emergency Response

PRESENTATION

DR. MICHAEL DOURSON: TOXICITY-BASED METHODOLOGY, THRESHOLDS AND POSSIBLE APPROACHES, AND UNCERTAINTY FACTORS

Present Toxicity-Based Methodology

In developing guidelines for deriving acceptable daily intakes (ADIs) for systemic toxicants, four types of response levels are considered:

NOEL:	No-Observed-Effect Level. That exposure level at which there are no statistically significant increases in frequency or severity of effects between the exposed population and its appropriate control.
NOAEL:	No-Observed-Adverse-Effect Level. That exposure level at which there are no statistically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control. Effects are produced at this level, but they are not considered to be adverse (e.g., the lowest NOAEL can be also termed a LOEL, that is a lowest-observed-effect level).
LOAEL:	Lowest-Observed-Adverse-Effect Level. The lowest exposure level in a study or group of studies which produces statistically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.
FEL:	Frank-Effect Level. That exposure level which produces unmistakable adverse effects, ranging from reversible histopathological damage to irreversible functional impairment or mortality, at a statistically significant increase in frequency or severity between an exposed population and its appropriate control.

Adverse effects are defined as any effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge. Frank effects are defined as overt or gross adverse effects (severe convulsions, lethality, etc.).

These concepts are illustrated in Figure 1. They have received much attention because they represent landmarks which help to define the threshold region in specific experiments. Thus, if an experiment yields a NOEL, a NOAEL, a LOAEL, and a clearly defined FEL in relatively closely spaced doses, the threshold region has been relatively well defined. Such data are very useful for deriving an ADI. On the other hand, a clearly defined FEL has little utility in establishing criteria when it stands alone, because such a level gives no indication how far removed it is from the threshold region. Similarly, a free-standing NOEL has little utility, because there is no indication of its proximity to the threshold region.

Based on the above dose-response classification system, the following guidelines for deriving criteria from toxicity data have been adopted:

- A free-standing FEL is unsuitable for the derivation of an ADI.
- A free-standing NOEL is unsuitable for the derivation of an ADI. If multiple NOELs are available without additional data, NOAELs or LOAELs, the highest NOEL should be used to derive a criterion.
- A NOAEL or LOAEL can be suitable for ADI derivation. A well-defined NOAEL from a chronic (at least 90-day) study may be used directly, applying the appropriate uncertainty factor. In the case of a LOAEL, an additional uncertainty factor is applied; the magnitude of the additional uncertainty factor is judgmental and should lie in the range of 1 to 10. Caution must be exercised not to substitute "Frank-Effect-Levels" for "Lowest-Observed-Adverse-Effect-Levels."
- If -- for reasonably closely spaced doses -- only a NOEL and a LOAEL of equal quality are available, then the appropriate uncertainty factor is applied to the NOEL.

In using this approach, the selection and justification of uncertainty factors are critical. The basic definition and guidelines for using uncertainty factors have been given by the National Academy of Sciences (NAS, 1977). "Safety factor" or "uncertainty factor" is defined as a number that

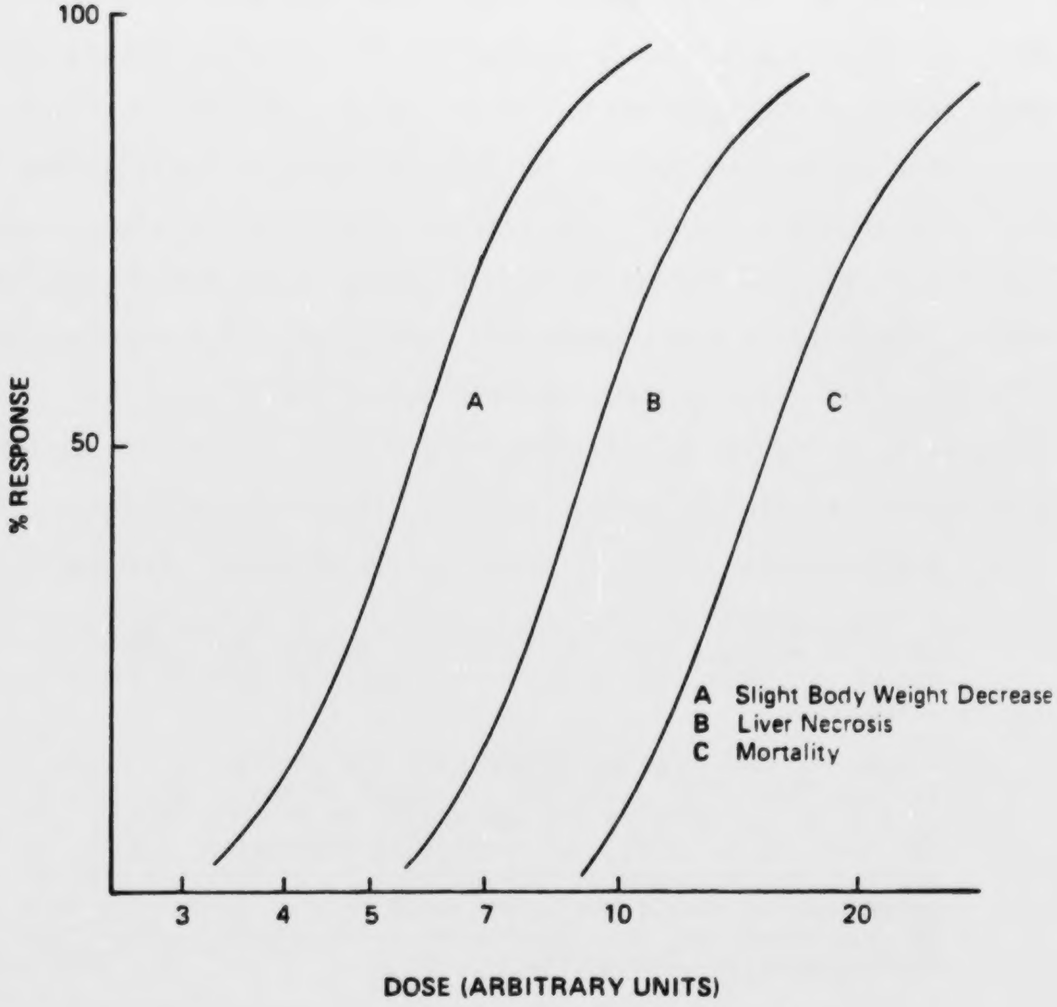


FIGURE 1

Response levels considered in defining threshold regions in toxicity experiments. Doses associated with these levels are as follows: 3 - NOEL; 4 - LOEL, NOAEL; 5 - NOAEL (Highest); 7 - LOAEL; 10 - FEL; 20 - FEL.

reflects the degree or amount of uncertainty that must be considered when experimental data in animals are extrapolated to man. Dourson and Stara (1983) discusses uncertainty factors in more detail.

Thresholds and Suggested Approaches

As part of my presentation, I would like to reference the following discussions by Drs. Clarkson, Crump, Hartung, O'Flaherty, Tardiff and the ECAO-Cin staff concerning thresholds and suggested approaches. These comments were made at previous ECAO meetings on risk assessment.

Dr. Thomas Clarkson:

In view of the large differences in toxicity, the nature of the toxic endpoint and the mode of action of toxicants, it is unreasonable to place all "non-carcinogens" in one category.

Categories should distinguish between reversible and irreversible action, between compounds that act rapidly on basic cellular process (e.g., cyanide) and those that have a delayed complex mode of action (skin sensitizers), and between compounds that have long biologic half-lives versus those that are rapidly eliminated.

Dr. Kenneth Crump:

The advantages and disadvantages of three options for estimating the health risk for systemic toxicants are discussed below.

Option 1:

Definition. Determine a NOEL or a NOAEL and apply a safety factor.

Advantages. This approach has been used for setting allowable exposure levels for many years. It is familiar to regulators, toxicologists and other scientists, and has been applied effectively to control human exposures to many substances.

Disadvantages. The NOEL approach does not fully utilize the slope of the dose-response curve. All other things being equal, a steeper dose-response should lead to higher safe levels. However the shape of the dose-response curve is disregarded in the NOEL approach except for deciding whether or not effects were observed at individual dose levels.

The size of the experiment is not fully incorporated into the NOEL approach. No observed adverse effects in larger numbers of animals represents greater evidence of safety and should lead to higher permitted exposure levels. However, this is not a part of the NOEL approach. Rather than rewarding good experimentation by the proponents of chemicals, it encourages them to use as few animals as possible, because with fewer animals adverse effects are less likely to be observed.

The NOEL approach also does not furnish particularly useful information for cost-benefit analyses; from a NOEL it is possible to estimate doses for which no effects are anticipated, but not the possible magnitudes of effects from exposures to higher doses.

Option 2:

Definition. Fit a mathematical model to dose-response data and, using statistical confidence limits, extrapolate downward to doses appropriately safe for humans (e.g., doses corresponding to risks of 10^{-5} or less).

Advantages. This approach has been used to a considerable extent in the past few years, chiefly for carcinogenic risks. It rewards good experimentation in that larger experiments tend to produce narrower confidence limits and consequently larger lower limits for safe doses. It also explicitly takes into account the shape of the dose-response curve because a mathematical model is fit to all of the dose-response data. It provides estimates of risk corresponding to any dose, along with associated confidence limits, and therefore can be conveniently used in cost-benefit analyses. The method does not, in principle, rule out thresholds, because a model which incorporates a threshold could be used for the extrapolation.

Disadvantages. The chief disadvantage of an extrapolation approach is related to the choice of the model; different models that fit the observed data equally well can yield vastly different results when extrapolated to doses corresponding to very small risks. With carcinogenic risks, information on the nature of the carcinogenic process is used to aid in the selection of a model. There are theoretical reasons to believe that the shape of the dose-response curve is apt to be approximately linear at low doses whenever a chemical initiates cancer through a change in the DNA of a single cell, or whenever background carcinogenesis is present. (This latter condition does not require carcinogenesis as the toxic response for its applicability.) Some experimental data from mutagenesis experiments also support the concept of low-dose linearity for genotoxic effects. The use by EPA and others of low-dose linear models for carcinogenic risk assessment reflects the point of view that the true dose response curve is likely to be linear in the low-dose region, and that curve shapes which predict appreciably higher risks than those predicted by a linear model seem very unlikely; thus it seems reasonable to calculate lower limits on safe doses from a model which is linear at low doses. The multistage and one-hit model used previously by EPA are linear at low dose.

For nongenotoxic events, a linear dose-response seems more unlikely than for genotoxic events. Although a linear model still would define upper bounds to low-dose risks, these upper bounds might overestimate the true risk by exceedingly large amounts in some instances. There are arguments which suggest that thresholds may exist in some cases. Thus the uncertainty as to the true shape of the dose-response curve for non-genotoxic effects and the fact that different dose-response curves can give vastly different results constitute a disadvantage to the extrapolation approach for non-genotoxic effects.

A second disadvantage to the extrapolation approach is that toxicological experiments are frequently not designed or reported in a manner which facilitates the use of model-fitting approaches. Frequently, the doses are selected too far apart to adequately describe the dose-response curve. Sometimes the data necessary for fitting a model are not reported in the open literature. However, the experimental design and reporting of data could be improved in future toxicological experiments once a model-fitting approach was adopted.

Option 3:

Definition. Fit a mathematical model to dose-response data and, using statistical confidence limits, calculate a lower confidence limit on the dose corresponding to a risk of 0.01; then apply a safety factor to this dose which reflects the severity of the toxic effect and the thoroughness of the toxicological study.

Advantages. This approach is intermediate between the first two options. It shares some of their advantages while avoiding some of their disadvantages. Like Option 2, but unlike Option 1, it takes the shape of the dose-response curve explicitly into account. It would reward good experimentation in that larger, better-designed experiments should yield higher lower confidence limits and thereby higher allowable human exposures. However, it would avoid much of the problem associated with Option 2 regarding the choice of the mathematical model for risk assessment. This is because there will be far less disagreement among various models if extrapolation is only carried out down to a risk of 10^{-2}. The size of the safety factor to be applied could then reflect the severity of the toxic effect, the thoroughness of the toxicological study, and possibly also information on mechanisms of action.

Disadvantages. This approach would share the disadvantage of Option 2 with respect to inadequate experimental designs and reporting of data.

Dr. Rolf Hartung:

Thresholds

Discussion of whether or not carcinogens or systemic toxicants elicit no response at some dose above zero (i.e., a practical threshold) centers primarily on what a threshold means biologically. Several scientists suggest that when xenobiotics act through a mechanism such as enzyme inhibition, depletion of required substances, or inhibition of transport mechanisms, then the production of an effect might be thought to depend on the interactions of: 1) the concentration of the xenobiotic; 2) the reaction with the receptor; 3) the reserve capacity of the affected system; and 4) the turnover time of the affected enzyme or the capacity of the repair system. All of these are mechanistically one step removed from the hypothesized mechanism of action for genotoxic carcinogens -- direct one-to-one interaction with DNA -- and therefore lead to a mechanism having a threshold.

Any reasonable health risk assessment approach should make use of as much of the available data as possible, and should also make use of the theoretical knowledge which has been accumulated in toxicology. This is exactly what enters into the so-called judgmental evaluations of a toxicologist, and in the following paragraphs I will try to outline some of these thought processes to make them more amenable to quantitative evaluations. The only responses which will be considered in this discussion are those that can be measured, recognizing that the visibility of a response is partially dependent on experimental design.

The biological responses to insult from foreign chemicals follow a series of progressions which can be presented as generalizations as follows:

1. At sufficiently low exposures for full life-times, no effect of any kind will be found in any tested organism, no matter how sophisticated the experimental design (this statement avoids theoretical considerations of the presence or absence of thresholds).
2. At higher concentrations for full life-times, subtle effects may be noted in a small proportion of exposed individuals. Such effects may be adaptive in nature, or may represent responses whose harmfulness is subject to honest debate. These concentrations are still sufficiently low, so that no experimental design can measure severely adverse effects in the exposed population. Similar circumstances can be produced by reducing the duration of exposure to less than life-time and increasing the concentration to offset the impact of shortened durations of exposure.
3. As the concentration-duration of exposure parameters increase, a greater proportion of the population will show the subtle effects noted under generalization 2, above, and a small proportion of the population will demonstrate effects which are slightly adverse. The early subtle effects have under many circumstances been considered to be "critical effects", meaning that if one protects the population from these early subtle effects, then no harmful effects will occur in the exposed population.

4. As the exposure parameters intensify, the incidence and severity of adverse effects in the population will increase. More and more of the population will become involved, and the occurrence of non-responders will decrease significantly as the exposure parameters increase. At some combination of exposure parameters, the entire measurable population will respond, some with more severe effects than others. Eventually even very simple experimental designs will indicate severe adverse effects in the exposed population in comparison to controls.

Suggested Approach

These known progressions of toxicologic responses form the basis of all judgmental evaluations of toxicologic risks. Operationally, this approach might entail the quantitative evaluation of all suitable animal and human data in terms of dose-response for a given exposure duration. The statistical model chosen for this evaluation needs to fit the experimental data with a minimum of parameters to be fitted, using a set of assumptions which are compatible with at least a portion of the biologic responses observed. For ease of computation, I would suggest the logistic model proposed by Berkson (1944). The question of ease of computation may become important, since I am proposing that all responses found in animals or humans which can be evaluated quantitatively should be formulated in terms of the logistic regression equation describing the response. The results would be a large set of regression equations for each chemical, describing the relationship of various exposure scenarios to response rates for a wide range of effects for each chemical. Knowing the exposure scenarios for various dumps, these regression equations could then be used to evaluate the likelihood that a specific exposure could result in a specific effect (subtle or otherwise) in a hypothetical test organism living on or near the dump site. It is likely that the incidence of subtle effects would be tremendously greater than the incidence of severe effects. When combinations of chemicals are evaluated, it may become obvious that a specific mix is likely to have a combined effect on one organ system, say the liver.

The evaluative scheme outlined above should not be construed as providing quantitative risk assessments, using the logistic model. Rather the scheme is intended to be used to uncover which dump site is producing the higher comparative risk, and what is the likely target organ site and effect at the exposure scenario which has been postulated as having occurred near that site. The intent would be to look for those effects in the exposed human population to uncover what the actual risk of sustaining subtle effects and untoward effects was. Even in the absence of any measurable effects the potential for adverse impact of various dump sites could be compared. Following such an approach has several advantages:

1. Dump sites, or other exposure sources, could be prioritized according to toxicologic responses found in animals or in humans, and policy decisions could be made on the basis of such data.

2. For the worst sites it may be possible to correlate animal responses with human responses. Although the occurrence of human effects would clearly represent a past failure of needed protective mechanisms, the evaluation of such events could provide opportunities for any adjustment of present regulatory approaches and allow evaluation of the scientific basis for risk assessment.

Dr. Ellen O'Flaherty:

Thresholds

The biological basis for the existence of thresholds for the action of systemic toxicants is simply that one molecule of a systemic toxicant is incapable of causing an adverse or even a measurable effect. On the other hand, one molecule of a genotoxic carcinogen is potentially capable of causing a transformation that will eventually be manifest as a tumor. This distinction is absolute. It is independent of considerations as to the efficiency of operation of detoxification and other protective mechanisms. It provides a firm conceptual basis for differentiating between threshold and non-threshold toxicants.

How, and whether, a threshold may manifest itself in an experimental study is a separate question. It leads directly to the concept of the operational or practical threshold which has been used by the U.S. EPA in developing the existing guidelines based on various no-observed-effect levels. There are several features of the no-observed-effect level that could be more fully developed here, however.

1. All these practical thresholds are dependent on the population size. The larger the study group size, the lower the highest NOEL is likely to be. This observation should influence the selection of a NOEL from among multiple available NOELs. As the guidelines are presently written, it does not.
2. There is a sequence of response levels, as recognized and discussed by the U.S. EPA. However, this sequence may vary with the organ or organ system under consideration. For example, an effect occurring early or at low exposure in the liver may have little relationship to development of an ultimately fatal nephropathy. The distinction between adverse and non-adverse effects is useful here, but is not sufficient. The critical effect should be clearly defined and its relationship to adverse and non-adverse effects discussed. The critical effect could be non-adverse, in the sense of the U.S. EPA's current definition of "adverse". For most compounds, there will be insufficient information to allow the critical effect or critical organ to be identified, and safety evaluation will have to fall back on classification of effects as adverse and

non-adverse. Nonetheless, the concept of critical effect is important, particularly since it relates directly to the issues surrounding risk assessment for lifetime versus partial lifetime exposure.

3. Irreversibility of effect is less important, from the standpoint of establishing a threshold level, than magnitude or severity of effect.

4. In spite of the conceptual distinction between threshold and non-threshold toxicants, thresholds observed in experimental studies with non-carcinogens may not represent "real" thresholds in hypothetical dose-response curves. At the relatively high doses used in toxicity studies, a threshold is likely to be observed in the dose range within which at least one critical protective mechanism is overwhelmed, abruptly altering the slope of the dose-response curve. In the scheme

   $$D \xrightarrow{1} C_B \xrightarrow{2} C_{RS} \xrightarrow{3} E,$$

   where D represents dose, C_B concentration in the blood, C_{RS} concentration at the receptor sites, and E effect, dose-disproportionate alterations at steps 1 or 2 could generate a practical threshold independent of the relationship between C_{RS} and E, or between C_{RS} and the fraction of the population exhibiting a specified effect. The observation that many practical thresholds are probably caused by shifts in the balance of absorption, distribution, elimination and detoxification mechanisms cannot, however, be used to support the thesis that "real" thresholds are illusory.

   One useful application here would be the identification of pharmacokinetic or other endpoints that could be monitored in humans and that might signal close approach to a threshold exposure range.

Suggested Approach

NOELs of various kinds are, and will continue to be, useful, especially where the mechanism of action and progression of effects are not well understood. The definition of adverse effects given by EPA is basically sound, and is sufficiently specific to provide guidance while allowing reasonable scope for scientific judgment. Continued application of uncertainty factors is justified on the basis that their past use appears to have provided protection. Table 1 from Dourson and Stara (1983) is a helpful inclusion.

Development of guidelines for estimating dose-associated risk to human populations on the basis of experimental animal data is not likely to be productive, in my opinion, because:

1. If an adverse effect (or, better, a critical effect) has been identified, it should be sufficient to act to prevent, as nearly as possible, the occurrence of that effect.
2. Any prediction of human response to systemic toxicants based on animal data and on our present understanding of dose-response relationships would be questionable at best. For the action of genotoxic carcinogens there is a model consistent with what is now understood about the mechanism of carcinogen action which, however imperfect it may prove to be and however it may require modification in the future, at least can be used to construct dose-response curves for human populations. Since the model does not include a threshold, in practical terms this means that we have a means of adjusting the slope on a species-by-species basis (by assuming that the mechanism is unchanged and adjusting the dose on the basis of body weight or surface area). For systemic toxicants there is no such model. The slope of the dose-response curve in the region of interest is thought to be determined by the range of

TABLE 1

Guidelines, Experimental Support and References for the Use of Uncertainty (Safety) Factors[a]

Guidelines[b]	Experimental Support	References[c]
1) **Use a 10-fold factor when extrapolating from valid experimental results from studies on prolonged ingestion by man.** This 10-fold factor protects the sensitive members of the human population estimated from data garnered on average healthy individuals.	Log-probit analysis; Log-probit analysis; Composite human sensitivity	Mantel and Bryan, 1961; Weil, 1972; Krasovskii, 1976
2) **Use a 100-fold factor when extrapolating from valid results of long-term feeding studies on experimental animals with results of studies of human ingestion not available or scanty (e.g., acute exposure only).** This represents an additional 10-fold uncertainty factor in extrapolating data from the average animal to the average man.	Body surface area/dose equivalence; Toxicity comparison between humans and rats or dogs	Rall, 1969; Evans et al., 1944; Hayes, 1967; Lehman and Fitzhugh, 1954
3) **Use a 1000-fold factor when extrapolating from less than chronic results on experimental animals with no useful long-term or acute human data.** This represents an additional 10-fold uncertainty factor in extrapolating from less than chronic to chronic exposures.	Subchronic/chronic NOAEL comparison; Subchronic/chronic NOAEL or LOAEL comparison	McNamara, 1976; Weil and McCollister, 1963
4) **Use an additional uncertainty factor of between 1 and 10 depending on the sensitivity of the adverse effect when deriving an ADI from a LOAEL.** This uncertainty factor drops the LOAEL into the range of a NOAEL.	LOAEL/NOAEL comparison	Weil and McCollister, 1963

[a] These factors are to be applied to the highest valid NOAEL or NOEL which does not have a valid LOAEL equal to or below it, in calculating an ADI when no indication of carcinogenicity of a chemical exists.

[b] Guidelines are in bold print. Guidelines 1 and 2 are supported by the FDA and the WHO/FAO deliberations (Lehman and Fitzhugh, 1954; Bigwood, 1973; Vettorazzi, 1976, 1980); Guidelines 1-3 have been established by the NAS (1977) and are used in a similar form by the FDA (Kokoski, 1976); Guidelines 1-4 are recommended by the U.S. EPA (1980).

[c] Table adapted from Dourson and Stara (1983). See this paper for references.

sensitivities of individual population members; the magnitude of this range varies with the toxicant. To undertake quantitative risk assessment, it would be necessary to stipulate both a threshold dose and a dose-response slope for humans. At the present time, lacking actual human data, we have no means of doing the latter. Data showing how (or whether) the slopes of dose-response curves in animal and human populations are related when the toxicant is the same could be very useful, but to my knowledge have not been tabulated.

Dr. Robert Tardiff:

The present approach to health risk estimation of systemic toxicants relies on four concepts related to response levels (i.e., NOEL, NOAEL, LOAEL and FEL) and applies uncertainty factors whose magnitude is determined by the quality of the data. Several additional aspects to this approach should be considered. First, there must be a recognition that the dose-response relationship is a continuum rather than a sequence of separate curves. Second, the analytical power of the NOEL and NOAEL is quite limited for three reasons: 1) toxicity studies utilize relatively few animals and, therefore, have relatively poor statistical sensitivity; 2) toxicity studies utilize genetically homogeneous individuals whose distribution of response is likely to be much narrower than that of the much more heterogeneous human population; and 3) none of the positive dose-response data are taken into account. Consequently, the NOEL and NOAEL are quite artificial and can only be considered operational thresholds of the experiment and are not to be confused with human population thresholds. Third, the entire dose-response curve for toxicants should be used rather than only a single point. That can be accomplished by using an approach that fits the data and can even be extended beyond the data points. For simplicity, the probit or logit models (which have been used extensively to structure dose-response relationships in biology in general and pharmacology in particular) should be utilized unless toxicologic mechanisms prescribe otherwise. Similarly, thresholds of acceptability or of risk toleration could be selected on the basis of the severity of the effects, again unless mechanistic data indicated the biologic threshold region in humans. Provided that there were some flexibility in selecting risk levels on the basis of severity of effect, this dose-response modeling approach would be far superior to the use of uncertainty factors.

ECAO-Cin Staff:

A possible approach to health risk estimation of systemic toxicants is to use a threshold multistage model to fit a chosen human or animal data set and to extrapolate this model to a 10^{-2} (1%) risk. The choice of one model over another does not really matter, since the majority of mathematical models give similar results at a lower 95% confidence limit (CL) on the dose associated with a 10^{-2} risk. A lower 95% CL on the maximum likelihood estimate (MLE) of the dose is then used for further adjustments to estimate an ADI. Implicit in this calculation is the assumption that systemic toxicants will elicit no response at some dose above zero. This may be regarded as a practical threshold.

The adjustments to the lower 95% CL are outlined and justified as follows:

1. Multiplication by (le/Le) x (Le/L) where le is the length of exposure, Le is the length of observation and L is the assumed lifespan of the mammal. These adjustments attempt to estimate an equivalent lifetime daily intake when exposure and observation are less than lifetime. These adjustments are used in a similar form when estimating an equivalent lifetime daily dose for genotoxic carcinogens. The justification for their use has been previously given (45 FR 79351-79352).

2. Division by the cube root of the body weight ratio, $\sqrt[3]{\frac{70}{w}}$, where 70 represents the assumed average adult weight and w the weight of the animal, accounts for differences in dose as measured in mg/kg body weight when dose as measured in unit of body surface area is assumed to be equivalent among species (Mantel and Schneiderman, 1975). This adjustment is also used for genotoxic carcinogens and is more fully described elsewhere (45 FR 79351).

3. Division by one or more uncertainty factors.* The magnitudes of these uncertainty factors can be justified categorically; together they can vary between 10 and 1000. These categories of uncertainty are:

   - The first area of uncertainty, associated with a value of 10, is justified by any lingering uncertainties in adjusting the response from animal data, both because of the wider variability in the human population when compared to the experimental animal, and because of differences in species sensitivity to adverse effects of a chemical. For example, the lower 95% CL reflects the sampling error on the MLE and the variability inherent in the experimental population. It does not represent the sensitive individuals and should not be misconstrued to be protective. The cube root of the body weight ratio assumes that dose, relative to body surface area, is equivalent among animals and humans. It does not account for any differences in variability or sensitivity among species to the adverse effects of the chemical. When human data are used, a dose reduction of 10 would still be advisable because of uncertainties in the exposure estimate.

   - A second area of uncertainty associated with a value of between 1 and 10 accounts for extrapolating from a projected 10^{-2} risk, which can be considered a LOAEL, to a comparable NOAEL as per EPA guidelines (45 FR 79353). Although a projected 10^{-2} excess risk derived by mathematical extrapolation is sufficiently low as to be undetectable in practical experimentation and, therefore, might be considered as a NOAEL in the classic sense of the acronym, such an incidence rate of adverse effects is clearly unacceptable in the human population and thus must be considered as an adverse effect level. The dose reduction of between 1 and 10, because of this category of uncertainty, should thus be thought to extrapolate from this projected effect level to a level which is below threshold, hence a no-effect level. Furthermore, the incidence extrapolations are sensitive to minor changes in the incidence data even at the 10^{-2} risk levels (although the CL is less sensitive than the MLE). A misclassified animal could lead to a higher projected 10^{-2} level and thus a higher ADI.

---

*Note: These uncertainty factors were developed solely for use of this procedure and are not to be confused with the standard uncertainty factors used for toxics.

However, certain data bases could be used to support the extrapolated estimate such that this category of uncertainty would be reduced. For example, if more than one good animal study in more than one species support the range of adverse effect and lack of effect at lower dose levels, one could assume threshold has been reached and reduce the value of 10 for this category of uncertainty accordingly.

- A third area of uncertainty associated with a value of between 1 and 10 reflects the degree of evidence of genotoxicity. EPA's Reproductive Effects Assessment Group (REAG) has classified the evidence of genotoxicity for different compounds into five areas. Below is a scheme that assigns different values of this uncertainty factor to different degrees of evidence for genotoxicity. Although the assignment of values is arbitrary, the approach seems reasonable in light of the uncertainties involved:

Positive	10
Suggestive	7
Inadequate	5
Inconclusive	3
Negative	1

One interesting aspect of this recommended approach is that if an uncertainty factor of 10 is assigned in this latter category because of positive evidence of genotoxicity, the end result is similar to the present methodology for carcinogens at a 10^{-5} risk level. The data of Kociba (1977) can be used to illustrate this point. During a 2-year hexachlorobutadiene feeding study, Kociba (1977) observed renal tubular adenomas and carcinomas in male rats with significantly higher incidence in animals fed 20 mg/kg/day than controls. Doses of 2.0 and 0.2 mg/kg/day showed no increase in tumor incidence. The dose of 2.0 mg/kg/day, however, elicited evidence of kidney toxicity in both male and female rats, whereas the dose of 0.2 mg/kg/day showed no evidence of toxicity in either sex.

The Kociba (1977) study served as a basis for estimating the ambient water quality criterion for hexachlorobutadiene using the linearized multistage model (45 FR 79351-79353) (i.e., the present method). The pertinent data are listed below:

Dose (mg/kg/day)	Incidence (No. responding/No. tested)
0.0	1/90
0.2	0/40
2.0	0/40
20.0	9/39

le (length of chemical exposure) = 669 days
Le (length of observation) = 730 days
L (assumed lifespan of animals) = 730 days
w (animal weight) = 0.610 kg
R (bioconcentration factor) = 2.78 ℓ/kg

With these parameters the carcinogenic potency for humans, q_1^*, was calculated to be 0.07752 $(\text{mg/kg/day})^{-1}$. As a result, the recommended ambient water concentration was 4.6 µg/ℓ in order to keep the individual lifetime risk below 10^{-5}.

The recommended procedure uses this data set and calculates a lower 95% CL of the dose rate associated with a 10^{-2} excess cancer risk† found with the threshold multistage model of 0.69 mg/kg/day. An ADI calculated from this procedure would be:

$$\text{ADI} = 0.69 \times \text{mg/kg/day} \times \frac{\left(\frac{669\ \text{day}}{730\ \text{day}}\right) \left(\frac{730\ \text{day}}{730\ \text{day}}\right)}{\sqrt[3]{\frac{70\ \text{kg}}{0.61\ \text{kg}}} \times 335} \times 70\ \text{kg}$$

$$\sim 0.027\ \text{mg/day}$$

---

†Excess cancer risk is used here in lieu of other systemic toxicity for illustrative purposes only. This procedure is not recommended for carcinogens.

where 669, 730 and 730 are the le, Le and L values in days as before, 70 and 0.61 are the respective weights for the average man and the rats in this study, and the 335-fold uncertainty factor represents a 10-fold because of area 1, a 6.7-fold due to area 2, and a 5-fold based on REAG's classification of the genotoxicity evidence as inadequate. This value can be used to determine a criterion by:

$$C = \frac{0.027\ \text{mg/day}}{2\ \ell/\text{day} + (0.0065\ \text{kg/day} \times 2.78\ \ell/\text{kg})}$$

$$\sim 0.013\ \text{mg}/\ell, \text{ or } 13\ \mu\text{g}/\ell.$$

If the evidence for the genotoxicity of hexachlorobutadiene was strong, an uncertainty factor of 10 instead of 5 in the ADI derivation would put the result in the range of the recommended ambient water quality criterion at a 10^{-5} excess lifetime cancer risk, i.e. $(13\ \mu\text{g}/\ell \times 5) \div 10 = 6.5\ \mu\text{g}/\ell$, as compared to $4.6\ \mu\text{g}/\ell$. If hexachlorobutadiene was considered not to be genotoxic, an uncertainty factor of 1 instead of 5 in the ADI derivation would result in an ADI of 0.136 mg/day and an ambient water quality criterion of $65\ \mu\text{g}/\ell$.

CRITIQUES

DR. THOMAS CLARKSON

Introduction

The purpose of this discussion is to seek ways of improving the traditional methods for calculating acceptable daily intakes. It is recognized that a new scientifically impeccable approach is beyond our reach at the present moment. Thus the emphasis is on improvement of the current methods and, indeed, not changing current procedures unless there are solid reasons.

"Pseudo-NOAELs"

Most of our discussion since the ECAO workshop on "Review of Guidelines for Ambient Water Quality Criteria for Carcinogens" in Washington, DC in February, 1982, led to agreement that all the positive data should be used. Crump has summarized the reasons for this: that is to say that a dose-response model will be used to calculate a NOAEL associated with a risk of 1%. Unfortunately, previous discussions have left the choice of the end-point somewhat arbitrary -- risk levels of 1%, 4%, or even 10% have been considered to define this "pseudo-NOAEL." At the last meeting an alternative approach was suggested -- to use segmented linear regression analysis. This will determine an "inflection" or "break" point where the effect due to the agent emerges above the background frequency. The dose associated with the "break" point is referred to as a "practical threshold" value and is equivalent to a "pseudo-NOAEL" (Figure 2). A probit or logit analysis that takes into account a background frequency indicates a risk level at the break point of about 4%. The segmented linear regression analysis has the advantage that it does not require an arbitrary choice of risk level.

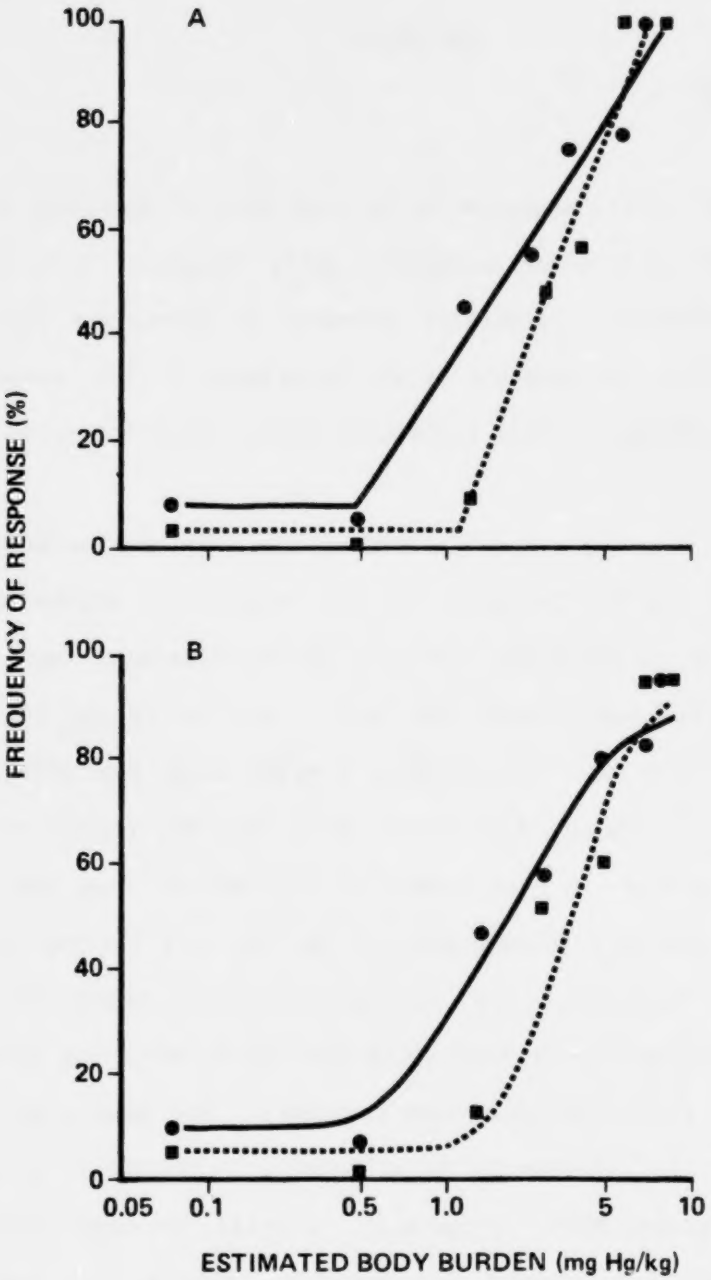


FIGURE 2

The frequency of signs and symptoms of methylmercury poisoning versus the maximum estimated body burden of methylmercury in adults. A. Data plotted according to "Hockey Stick" method. B. Data plotted according to logit analysis.

Source: Data taken from Bakir et al., 1973 (Copyright permission granted)

Statistical models are well developed for segmented linear regression. The confidence limit for the break point value can be calculated.

I disagree with the claim of the ECAO-Cin staff that the "pseudo-NOAEL" is a LOAEL. We are dealing with animal data at this point, and a 4% risk is below a measurable value.

Extrapolation to Man

The question arises whether the maximum-likelihood estimate of the "pseudo-NOAEL" or its 95% lower limit be used as the starting point for extrapolation to man. The latter would have the merit of taking into account the quality of the data.

Expression of the dose in units of surface area rather than the traditional units of body weight does not seem to be well justified. Very little data exist to indicate that surface area conversions reduce interspecies differences. In fact, for extrapolations from mice and rats, the use of the surface area units creates a safety factor of 10 over units based on body weight. For larger animals, this "surface area" safety factor would be less and could actually be less than unity, even though there is no guarantee that larger animals are more similar to man than small rodents.

Dourson (1982) has summarized the rationale for the use of safety factors and has reviewed evidence supporting the magnitude of these safety factors. For interspecies conversion, i.e., from all animals to man, a maximum value of 10 would appear to be appropriate. This factor has been designated 10_1 by Dourson. In the absence of relevant information, this factor would normally be used. However, if evidence exists that a certain species is similar to man for a given chemical, expert judgment should be used in choosing the actual value.

A maximum value of 10 also seems appropriate for a second safety factor to cover differences in human susceptibility. This has been designated 10_2 by Dourson. He has summarized evidence from the literature concerning the distribution of LOEL values in human populations to indicate that a factor of 10 would cover most of the variance in human threshold values. Again, in the absence of other information, the maximum value of 10 would normally be used. However, if dose-response data or mechanistic data indicate a narrowed distribution of threshold, the actual value could be less than 10 based on expert judgment.

The possibility of applying a third safety factor of 10, 10_3, has been discussed by EPA and others. The idea is to take into account the possibility of other factors and uncertainties not covered by the first two factors, such as the quality of the data, the duration of the study, and even the severity of the effect. However, the need for this safety factor might be avoided in many cases if the lower 95% confidence limit in the NOAEL is used as the starting point for extrapolation to man.

DR. HARRY SKALSKY

As Dr. Dourson and Dr. Clarkson have discussed, the quantitation of a safe dose is a difficult task that requires considerable judgment. As toxicologists, we are constantly aware of a paucity of proven scientific facts concerning safety assessment. It is satisfying that Dr. Dourson's paper has demonstrated a biologic basis for our traditional safety factor approach.

There are two distinct areas of quantitation being discussed: the statistical issues surround the shape of a particular dose-response curve, and the precision involved in extrapolating from animal to man.

There are three basic pieces of information that may be gleaned from a proper dose-response experiment.

No-Observable-Effect Level (NOEL)

The NOEL may be practically defined as the point at which the measured response can no longer be distinguished from the controls. This is a statistically measurable entity that can, as Dr. Dourson has discussed, be determined.

Margin of Safety

This term may be defined as the magnitude of the range of doses involved in progressing from a no-effect dose to the maximum effective dose. In general, the slope of the dose-response curve may be considered an "index" of the margin of safety. It provides one with a general indication of NOEL's "resistance" to change if a particular experiment is repeated.

Comparative Toxicity

Compounds may be ranked or compared by their relative activity within a uniform biological specimen. As you are aware, the traditional LD_{50} (Litchfield and Wilcoxon, 1949) approach has proven to be very valuable in distinguishing the relative toxicities of a great variety of compounds.

Obviously, there are a great many mathematical ways to depict dose-response data. It is always prudent to remember that dose-response data originates from a cumulative frequency distribution which may be unique. Observers sometimes allow these mathematical manipulations to extend their conclusions beyond the scope of biological data. There are many biologic observations intrinsic to the animal bioassay that are not expressed by the dose-response curve.

The mathematical alternatives (multi-hit/safety factor approach) being considered by EPA do not appear to offer any clear advantage over the traditional NOEL approach. Since there are many biologic observations intrinsic to the animal bioassay that are not expressed by the dose-response curve, the EPA alternative factor might obfuscate the professional judgment that has been an integral part of the NOEL-safety factor process. At present, there is no theoretical basis for the use of a non-threshold model in calculating no-effect levels for noncarcinogenic chemicals. Thus, on scientific grounds, any serious consideration of the mulsti-state alternative is not warranted. In the practical sense, it would not be prudent to replace the traditional NOEL-safety factor approach with a "novel" model that offers no substantial advantages.

If advancements are to be made, we must not dwell on the manipulation of dose-response data. Instead, we must better address the second area of quantitation: the extrapolation from animal to man. Success in this quantitation can be measured directly by the ability to predict human responses from animal data. It is in this area that toxicology has obviously lagged behind the science of pharmacology.

As you can see (Table 2), there are a large number of physiologically based pharmacokinetic models that have attempted to quantify the interspecies issue in their prediction of various drug effects. Each of these models has addressed the animal-to-man issue with various mathematic assumptions. The accuracy of some of these models can be illustrated by Figure 3. The solid lines on this graph represent mathematic predictions of human serum concentrations of cytosine arabinoside (ARA-C) and its metabolite (ARA-U) based only on animal and in vitro experiments. These predictions were made prior to the collection of human data. However, as you can see when the human experiment was performed (graphically depicted by dots and triangles), the predictions were very good.

TABLE 2

Physiologically Based Pharmacokinetic Models*

Drug	Species	Reference
Thiopental	dog, human	Bischoff, 1968
Methotrexate	mouse, rat, man	Bischoff, 1970, 1971; Dedrick, 1973, 1975, 1978
Cytarabine (ARA C)	mouse, monkey, man	Dedrick, 1973, 1978; Morrison, 1975
Adriamycin	rabbit, man	Harris, 1975
Cyclocytidine	man	Himmelstein, 1977
Digoxine	rat, man	Harrison, 1977
Ethanol	man	Dedrick, 1973
Mercaptopurine	rat, man	Trerlikkis, 1977
Lidocaine	monkey, man	Benowitz, 1977
Sulfobromophthalein	rat, man	Chen, 1978

*Source: Adapted from Himmelstein and Lutz, 1979 (Copyright permission granted)

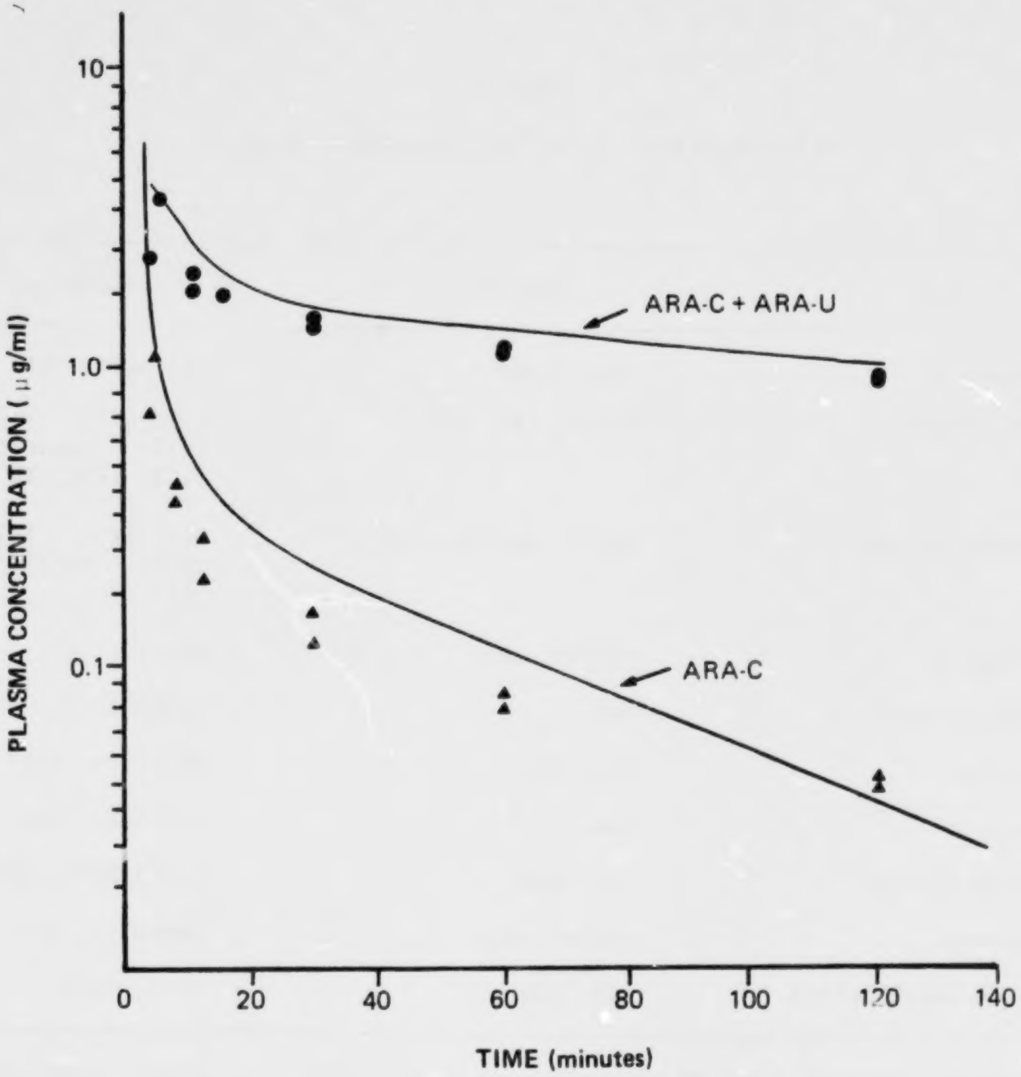


FIGURE 3

Predicted human serum concentrations of ARA-C and its metabolite ARA-U compared with experimental data. (All kinetic parameters based on *in vitro* work; all anatomic and physiologic parameters based on animal data.)

Source: Dedrick, 1973 (Copyright permission granted)

The type of data that these models utilized (Table 3) has been available for some time and it is apparent that we toxicologists have not made full use of it. Most of the comparative anatomic and physiologic data have been available since the late 1940s (Adolph, 1949; Guyton, 1947, etc.). The thermodynamic and transport data have been more recent developments as have the perfusion techniques (Wiersma and Roth, 1980; Rane et al., 1977), which have provided important information on tissue-specific metabolism.

The physiologically based pharmacokinetic models are constructed by compartmentalizing the basic biologic data (Figure 4). Then the mathematic equations are constructed to explain each compartment and their interrelationships. As you can see, some of these models can become quite complex.

The complexity and sophistication of these models appear to be limited only by the available data. For example, Roth and Weirsma (1979) have attempted to predict the comparative clearance of benzo(a)pyrene (Figure 5) from tissues (liver and lung) in the basal and the induced metabolic state. If such complex metabolic relationships can be predicted, the future of these models looks bright.

In summary, I would like to offer the following three comments: First, I believe that any ADI established by EPA can result directly or indirectly in a "regulatory" number. For this reason, a minimum data base for setting an ADI must be defined. For a noncarcinogenic endpoint, no less than a well-designed 90-day study should be acceptable. This concept was not included in any of the meeting materials and is not on the agenda for discussion. I believe, however, that it is necessary for EPA to address this issue and, perhaps this group of scientists can aid in that decision.

TABLE 3

Types of Data Utilized by Physiologically Based Pharmacokinetic Models

---

1. Comparative Anatomical Data Between Species:
    a. Organ Sizes
    b. Tissue Volume
2. Comparative Physiologic Data:
    a. Blood Flow, Urine Output, Ventilation Rate, etc.
    b. Basal Metabolism
    c. Compound Metabolism
3. Thermodynamic Data:
    a. Protein Binding
    b. Tissue Storage
4. Transport Data:
    a. Membrane Permeability
    b. Tissue Perfusion

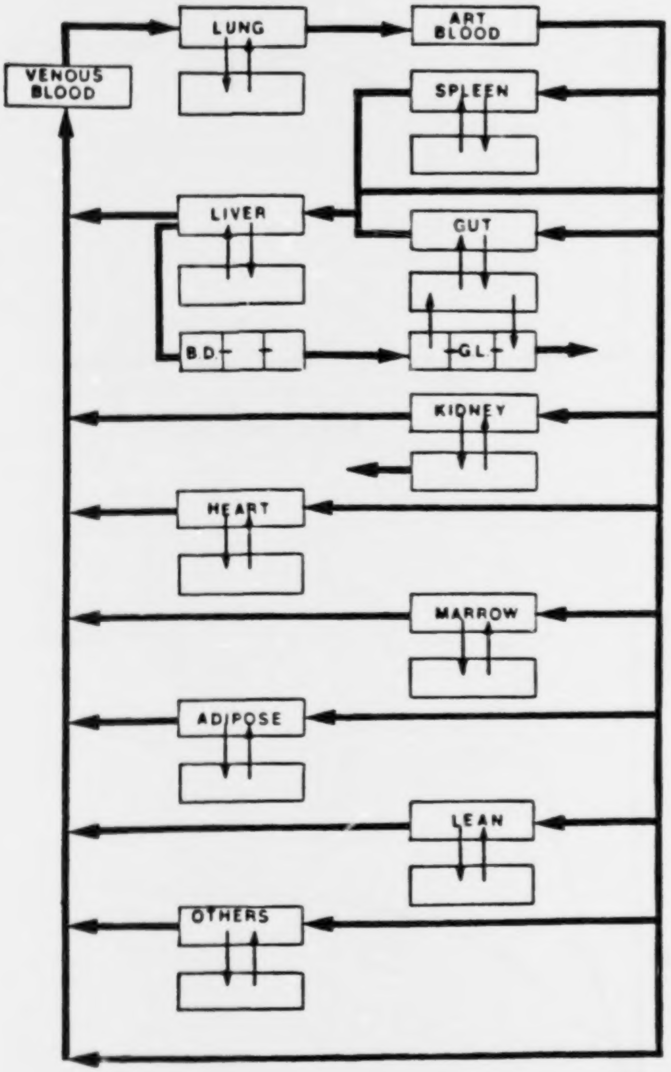

LUNG
ART BLOOD
VENOUS BLOOD
SPLEEN
LIVER
GUT
B.D.
G.L.
KIDNEY
HEART
MARROW
ADIPOSE
LEAN
OTHERS

FIGURE 4

Physiological Schema for Pharmacokinetic Modeling

Source: Himmelstein and Lutz, 1979 (Copyright permission granted)

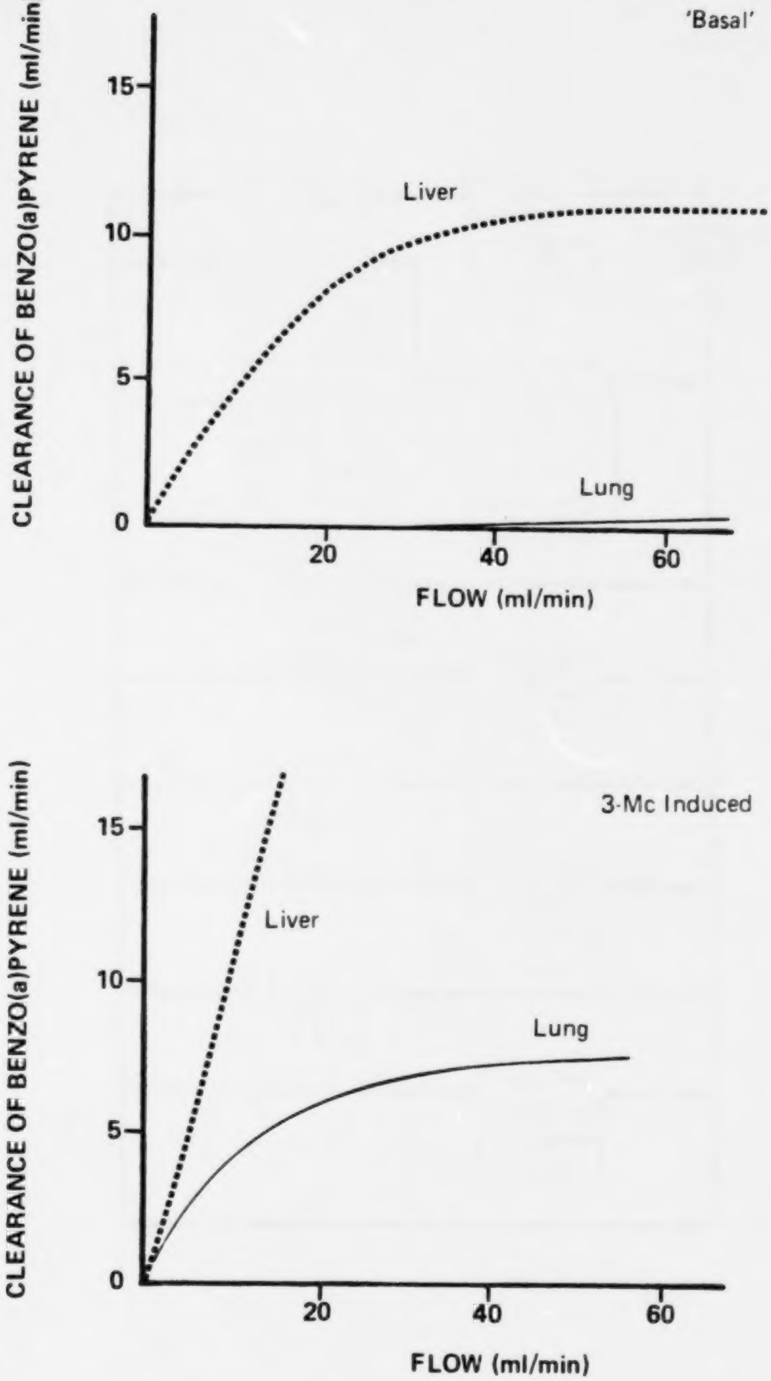


FIGURE 5

Predicted Clearance of Benzo(a)pyrene

Source: Roth and Wiersma, 1979 (Copyright permission granted)

Second, the traditional NOEL-NOAEL-safety factor approach at setting ADIs is clearly the best of the options being presently considered by EPA. Third, it is obvious that the pharmacologists have a headstart on us at being able to predict drug effects in man based on animal data. However, there is no reason why these physiologically based pharmacokinetic models would not also be successful at predicting potential toxic effects of environmental chemicals. The need for these predictive models is clear. Perhaps now is the time for the Agency to explore these models so that their goals of prediction can be fulfilled in the future.

DR. ARTHUR PALLOTTA

Most of this presentation's emphasis has been on pesticides and drugs. However, most of the chemicals that the Solid Waste Emergency Response Office must deal with are industrial chemicals, and the data base for these chemicals is poor.

Setting minimum data base requirements is an excellent recommendation. For example, trichloroethylene has been found in 40% of all dump sites, but different offices used different data to calculate an ADI, resulting in a dilemma, i.e., which ADI should be chosen.

DISCUSSION

DR. MYRON MEHLMAN

This terminology (i.e., acceptable daily intake) and some of its underlying assumptions should be reassessed and strengthened. No exposure to foreign or synthetic chemicals is acceptable. It is of no benefit whatsoever to the person being exposed. Thus the term should be changed to "no adverse effect from daily intake."

DR. ROBERT TARDIFF

Development of acceptable daily intakes (ADIs) for substances in waste dumps is implausible for several reasons. First, ADIs deal with compounds individually and do not take into account interactive effects (e.g., additivity and potentiation) from exposures to multiple agents. Second, ADIs imply virtual safety, when in fact some degree of risk is likely to exist, and they do not truly account for differential potency of the various substances. Third, ADIs do not allow for the array of data so that comprehensive decisions can be made, i.e., there could be no organization by quantitative risk estimates such as numbers of substances with specified risk levels, and no organization by qualitative risk such as the assembly of compounds toxic to any individual target organ such as the central nervous system. A more plausible approach for the simultaneous health risk analysis of a variety of substances is the uniform application of quantitative risk assessment methodology (similar in concept but not necessarily in detail to that applied to carcinogens) to obtain risk estimates for individual toxic endpoints for each substance or mixture. Such uniformity of data should allow for a more logical selection of critical adverse effects and of risk levels that have been determined elsewhere to be socially acceptable. By arraying the data according to target organ risk (or even by mechanisms of action if known), there would be at least a hypothetical basis for anticipating the additivity of risks and for selecting classes of acceptable risks for specific waste disposal sites.

MR. WILLIAM GULLEDGE

The presentation by Dr. Harry Skalsky was most illuminating and should be supported. The safety factor approach, properly implemented, is a definite improvement over the use of complex risk assessment models.

DR. MAGNUS PISCATOR

The statement by Crump that the slope should be used is basically sound. However, occasionally a steep slope may depend on additive effects. As an example: if a nephrotoxic agent also causes hemolysis, a steep dose-response curve may be obtained for renal effects within a certain dose interval. If lower doses are used and no hemolysis occurs, the dose-response curve for renal effects may not be so steep, leading to a lower safe level. This also implies that all effects must be taken into account when looking at the dose-response curve for one effect. This is interaction of effects, which was not mentioned at the meeting except in my comment.

DR. HARRY SKALSKY

It is important that a minimum data base be established with which to calculate an ADI. For a noncarcinogenic endpoint, no less than a well-designed 90-day study should be acceptable. The traditional NOEL (NOAEL)-safety factor approach at setting ADIs is clearly the best of the options that EPA is currently considering. Perhaps the physiologically-based pharmacokinetic models will provide new ideas with which to improve the process of safety evaluation.

DR. RICHARD KOCIBA

Conceptually, there is considerable merit in the use of various safety factors (uncertainty factors) in estimating acceptable daily intakes for chemicals. While historically this has been most frequently used in dealing with noncarcinogenic endpoints, newer scientific information now supports the use of safety factors in dealing with carcinogenic endpoints, especially endpoints of an epigenetic type.

This would allow one to more fully utilize all the data available in setting more realistic levels of control. A paper by Park and Snee (1982) illustrates one option that should be considered.

The definitions of NOEL and NOAEL should be revised to give equal weight to the biological significance in addition to the statistical significance.

It is not always appropriate to categorically assume that man is going to be 13 times more sensitive than the mouse and 5 times more sensitive than the rat as based on body surface area ratio. This concept has been based on alkylating agents, and is not supported by data from other materials. A paper by Reitz et al. (1978) pertains to this issue. It would be more appropriate to deal with each material on a case-by-case basis, and use mg/kg body weight as the basis of interspecies conversion where appropriate.

GENERAL COMMENTS

- The FEL could be predicted for untested chemicals from structure-activity models.
- The lack of data is the driving force for making the safety factor as low as possible.
- There should be a minimum data base requirement before making ADI calculations. In the absence of these data, a more severe adjustment should be made.
- Minimum study quality parameters should be formally set.

- Since data for multiple exposure to chemicals do not exist, as rigid a standard as possible should be established in view of current technology, in the hope that if the standards are difficult or burdensome to achieve, they will lead to the development of the necessary data.

- The degree of exposure should dictate whether minimum data are used.

- Physiologic pharmacokinetic models should be explored. Caution should be used with these models until molecules can be measured at the site of toxicity.

- Kinetic data on key, commonly occurring chemicals can be used to develop the necessary equations for individual compounds to predict the likelihood of unusual reactions due to interactions.

- Monitoring data are needed to establish exposure levels.

- An uncertainty factor representing the quality of data could be used for data taken from an uncertain data base.

- Significant biologic bases should be evaluated as well as statistical significance in using the ADI approach. Physical and biologic data should be used.

- Use of surface area adjustment may be a problem, since pathologists will report severity data and not incidence data.

- Surface area adjustment implies that children and infants can tolerate higher doses than adults.

- Risk assessments should be validated and updated when additional information is available.

- Animal data can't always be considered reliable, e.g., neurobehavioral aspects can't be determined in animal models.

- Regulatory agencies should aim at setting standards that will prevent us from getting human effects data.

- A discussion of risk assessment should deal with predictive methodology, not protective methodology.

- The ADI approach will establish an overly simplistic situation of whether a dump site is safe or unsafe.

REFERENCES

Adolph, E.F. 1949. Quantitative relations in the physiological constitutions of mammals. Science. 109: 579-585.

Andersen, M.E. 1981. Saturable metabolism and its relationship to toxicity. CRC Crit. Rev. Tox. p. 105-149, May.

Bakir, F., S.F. Damluji, L. Amin-Zaki, et al. 1973. Methylmercury poisoning in Iraq. Science. 181: 230-241. (Copyright 1973 by the AAAS)

Berkson, J. 1944. Application of the logistic function to bioassay. J. Am. Stat. Assoc. 39: 357-365.

Bischoff, K.B., R.L. Dedrick, D. Zaharko and J.A. Longstreth. 1971. Methotrexate pharmacokinetics. J. Pharm. Sci. 60: 1128-1133.

Dedrick, R.L. 1973. Animal scale-up. J. Pharm. Biopharm. 1(5): 435-461.

Dedrick, R.L. and K.B. Bischoff. 1980. Species similarities in pharmacokinetics. Fed. Proc. 39: 54-59.

Dourson, M.L. and J.F. Stara. 1983. Regulatory history and experimental support of uncertainty (safety) factors. Reg. Tox. Pharm. (In press)

Guyton, A.C. 1947. Analysis of respiratory patterns in laboratory animals. Am. J. Physiol. 150: 78-83.

Harrison, L.I. and M. Gibaldi. 1977. Physiologically based pharmacokinetic model for digoxin distribution and elimination in the rat. J. Pharm. Sci. 66: 1138-1142.

Himmelstein, K.J. and R.J. Lutz. 1979. A review of the applications of physiologically based pharmacokinetic modeling. J. Pharm. Biopharm. 7: 127-145.

Kociba, R.J., et al. 1977. Results of a 2-year chronic toxicity study with hexachlorobutadiene in rats. Am. Ind. Hyg. Assoc. 38: 589.

Litchfield, J.T. and F. Wilcoxon. 1949. Simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99-113.

Mantel, M. and M.A. Schneiderman. 1975. Estimating "safe" levels: A hazardous undertaking. Cancer Res. 35: 1379-1386.

NAS (National Academy of Sciences). 1977. Drinking Water and Health. NAS, Washington, DC.

Park, C. and R. Snee. 1982. Quantitative Risk Assessment: State of the Art for Carcinogenesis. (Submitted for publication)

Rane, A., G.R. Wilkinson and D.G. Shand. 1977. Prediction of hepatic extraction ratio from in vitro measurement of intrinsic clearance. J. Pharmacol. Exp. Therap. 200: 420-424.

Reitz, R., P.J. Gehring and C. Park. 1978. Carcinogenic risk estimation for chloroform: An alternative to EPA's procedures. Food Cosmet. Toxicol. 16: 511.

Roth, R.A. and D.A. Wiersma. 1979. Role of the lung in total body clearance of circulating drugs. Clin. Pharm. 4: 354-367.

Wiersma, D.A. and R.A. Roth. 1980. Clearance of 5-hydroxytryptamine by rat lung and liver: The importance of relative perfusion and intrinsic clearance. J. Pharmacol. Exp. Therap. 212: 97-102.

SYSTEMIC TOXICANTS

Interspecies Conversion of Dose and Duration of Exposure

Presentation:	Dr. Rolf Hartung University of Michigan
Critique:	Dr. Robert Tardiff National Academy of Sciences
Critique:	Dr. Ellen O'Flaherty University of Cincinnati

PRESENTATION

DR. ROLF HARTUNG: INTERSPECIES CONVERSION OF DOSE AND DURATION OF EXPOSURE

Accounting for Species Differences

Simple observation demonstrates that species differ in size, food habits, metabolic patterns, lifespan, and anatomical features. All these factors may influence the relative sensitivity of various species to chemicals. The toxicity of chemicals to various species may be compared on several bases.

The most common is in terms of mg/kg of body weight. This assumes that since the biochemical make-up of various species is very similar, the chemical should interact on the basis of its concentration within the organism. However, most laboratory animals tested have been shown to have a higher metabolic rate than man. Similarly, smaller animals have been shown to have higher rates of food intake, higher water consumption, higher breathing and heart rates, higher rates of excretion, and possibly higher rates of drug metabolism than larger animals.

Since the basic metabolic rate of homeotherms correlates well with body surface area, the comparison between species might be made on the basis of mg/m^2 of body surface. This is the currently accepted methodology used by the Agency in establishing water quality criteria. One difficulty in using this approach is that, for accurate predictive purposes, it may be necessary to know whether the metabolic processes predominantly detoxify the chemical or activate it to a toxic metabolite. If one also considers species differences in metabolic patterns, which may have nothing to do with body size, then a very complex pattern emerges. In developing criteria, we may need to pay much more attention than we previously have to the differences or similarities in the metabolic patterns of different species.

If one investigates the problem within one species only, e.g., the differences in drug sensitivities between children and adults (Wagner, 1971), then a number of problems become evident. In general, the child is less sensitive to drugs than the adult on a mg/kg basis, and the best approximator of the appropriate dose for children is:

child's dose = adult dose (body surface area of child/
body surface area of adult).

In this case the appropriate dose for the smaller organism is predicted from our experience with the larger organism, and this approach is compatible with the general application of the body surface rule currently used by the Agency. However, Wagner also makes the observation that the dosing regimens for newborns cannot be predicted, because of differences in the development of various enzyme systems.

Other means of comparing effects among species have also been suggested. Thus Harwood (1963) suggested using mg/kg brain weight, presumably for the evaluation of chemicals with CNS activity.

Accounting for Differences in Duration of Experiment and Lifespan

In carcinogenic bioassays, it has usually been assumed that the induction time for cancers over the lifetime of a relatively short-lived rodent is equivalent to the relatively longer induction time over the lifetime of longer-lived animals such as humans. Thus the duration of an exposure has sometimes been represented in terms of the proportion of that exposure relative to lifespan (t/T). The extent to which that concept is valid may require investigation.

Shorter and longer exposures have often been compared on the basis of Haber's rule (straight time-weighted dose averaging). Haber's rule appears to be applicable as an approximation when only slight differences in dose or duration are involved. A more complex relationship, reviewed by Filov (1979), may be more promising.

When one compares relatively acute or subacute phenomena, species conversions on the basis of lifespan may have no applicability whatever. The time to develop liver necrosis or enzyme changes appears to be very similar in man or in mouse. Thus, we need to better review the available knowledge, or to generate more basic data to enable us to be more certain whether and what kind of temporal relations exist.

CRITIQUES

DR. ROBERT TARDIFF

The previous presentation reviewed data manipulation techniques. However, none of the techniques presented has been sufficiently validated to encourage their broad-based application. An additional aspect, not covered in the narrative, is the expression of dose as moles rather than as weight of a compound. The use of moles would provide a more accurate comparison of potencies of chemicals, for it describes the number of molecules required to induce adverse effects in an organism. Since differential potency is of considerable importance in predicting the health risks from these mixtures, such an approach is far more desirable for chemical waste dump evaluations. With regard to adjustment for duration of exposure for noncarcinogens, the differences in potency between subchronic and chronic exposures are generally negligible -- if Weil's data (Weil and McCollister, 1963; Weil et al., 1968) are to be believed. One exception would be for substances that take longer than 90 days of exposure to reach equilibrium (e.g., methyl mercury); then subchronic data would not be predictive of chronic effects. This would argue strongly for the use of metabolic data to determine whether to adjust for duration and, if so, by what magnitude. By contrast, for initiating carcinogens the influence of duration of exposure on expression of disease must almost of necessity be obtained empirically because the primary lesion is likely to be acute. For substances that are unequivocally only promoters, less than lifetime exposure would be expected to have a threshold-curvilinear effect on cancer manifestation (i.e., 10% lifetime exposure is likely to have less than 10% risk of cancer assuming a standard dose rate).

DR. ELLEN O'FLAHERTY

Interspecies Conversion of Dose

The method of expressing dose on the basis of body surface area has been widely accepted for two reasons: the good correlation of basal metabolic rate with body surface area, and the work of Pinkel et al. (1958) and Freireich et al. (1966) showing that, for chemically different antineoplastic agents, the maximum tolerated dose in several different species was about the same when expressed on a body surface area basis, but varied widely when expressed on a body weight basis. However, with regard to this particular class of drugs, Dixon (1976) has shown, using data from Feireich et al. (1966) and Schein et al. (1970), that the ratios between maximum tolerated doses of more than 30 antineoplastic agents in different species were reasonably constant from drug to drug, regardless of whether they were expressed on the basis of body weight or body surface area.

Wagner (1971) carried out a comparable evaluation of a number of drugs that apparently were not anticancer agents, using blood level parameters rather than toxicity data to assess comparability of exposure. Unfortunately, he did not identify the drugs that were included in his evaluation. However, he concluded that:

> Because of the high correlation between calculated body surface area and body weight, and the high correlation between blood level parameters (such as area under the curve or peak blood level) and dose by weight, it would be extremely difficult, if not impossible, with any given drug to prove that a blood level parameter correlates significantly better with dose per unit body surface area or dose per unit body weight. The data indicated that the choice between mg/kg or mg/m² correlation would be equivocal with any given drug.

It appears probable that this issue is one that is not resolvable on a scientific basis, no matter what the quality of the data base.

Duration of Exposure

It is interesting to note that the maximum tolerated doses used by Dixon (1976) in his interspecies comparisons were calculated by time-weighted averaging. Certainly this is the simplest method by which dose may be adjusted for duration of exposure. There is also a simple variant of time-weighted averaging that takes into account the possibility that an apparent threshold dose exists and/or that there is a minimum time to occurrence of the earliest observable effect.

Straight time-weighted averaging is illustrated in Figures 6 and 7, where X can be either dose rate or time and Y is the other (i.e., the expression is symmetrical with respect to dose rate and time). In an arithmetic coordinate system this expression plots as a family of hyperbolas whose shape depends only on the value of C; that is, on the total dose. Plotting in a log-log coordinate system (see Figure 7) produces parallel straight lines whose slope is -1.

If threshold dose and minimum time to effect are incorporated into the expression for total dose (as A and B in Figure 8), the family of hyperbolas is simply shifted with respect to the axes of an arithmetic coordinate system. However, it no longer plots as straight lines in a log-log coordinate system (Figure 9), although the slope is still -1 at the midpoint. The log-log plot is the one recommended by Filov et al. (1979), possibly on the basis that it produces straight lines that can be extrapolated to facilitate interconversions between dose rates. Filov et al. state, "Comparison of concentration-time relationships for various substances has shown the slopes to be different for different substances." As Figure 9 shows, while plots of segments of these lines might appear to be linear in a log-log coordinate system, the slopes of these segments will be determined by how closely the

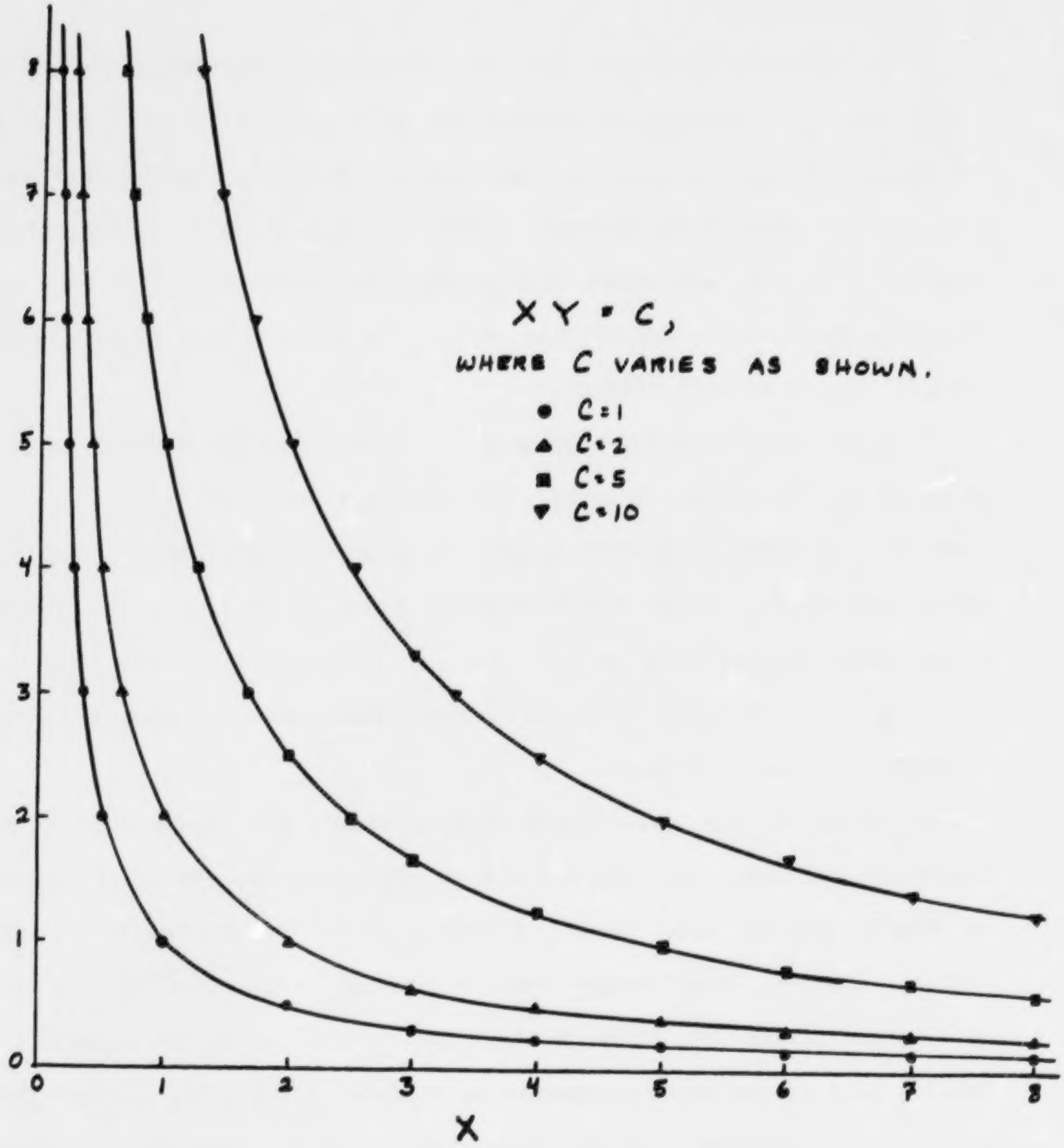


FIGURE 6

Time-weighted dose averaging using an arithmetic coordinate system. Plotting in an arithmetic coordinate system produces a family of hyperbolas showing the interrelationships of dose rate and time for four different total doses C.

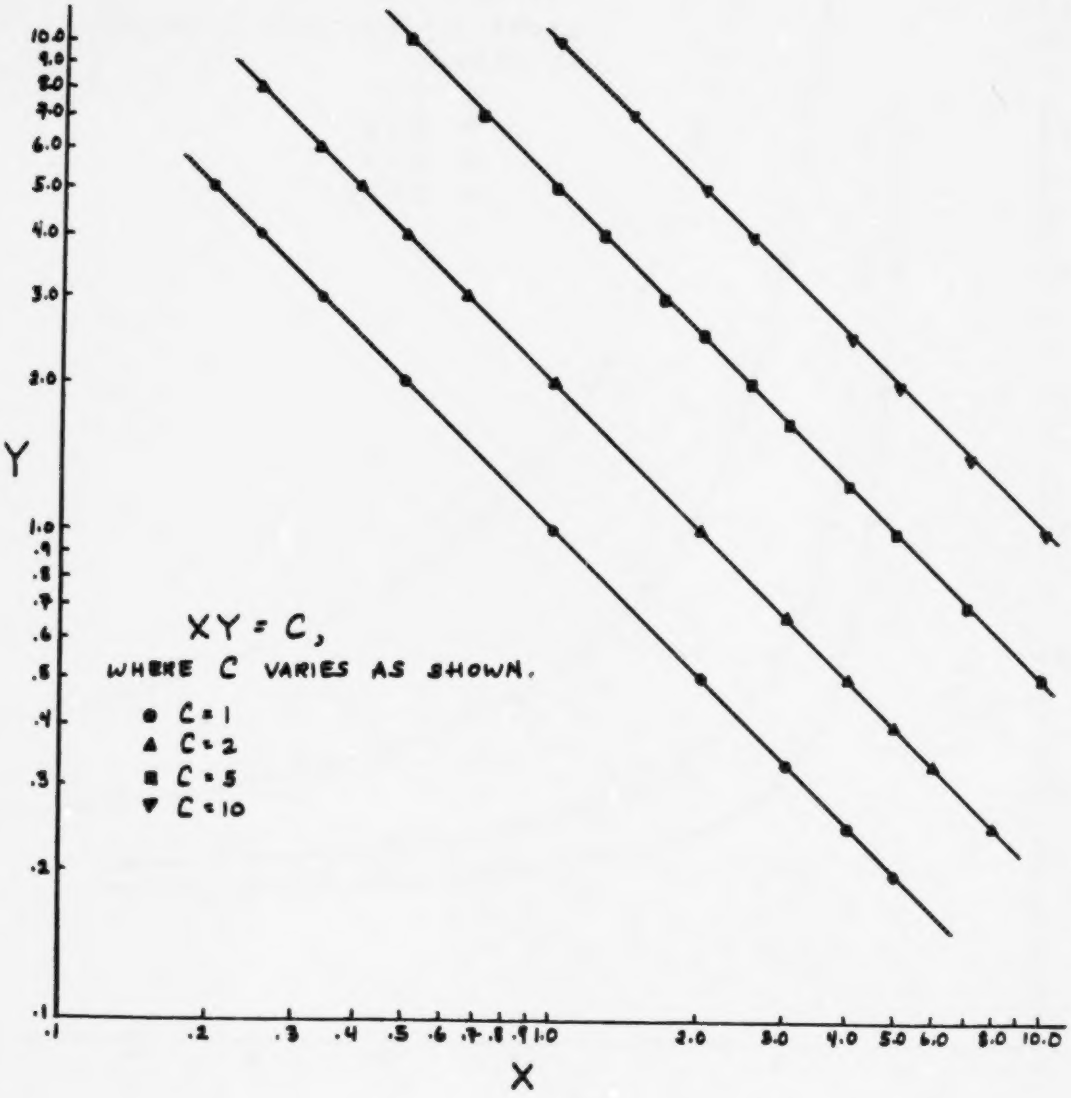


FIGURE 7

Time-weighted dose averaging using a log-log coordinate system. Plotting the data from Figure 6 in a log-log coordinate system produces a family of parallel straight lines.

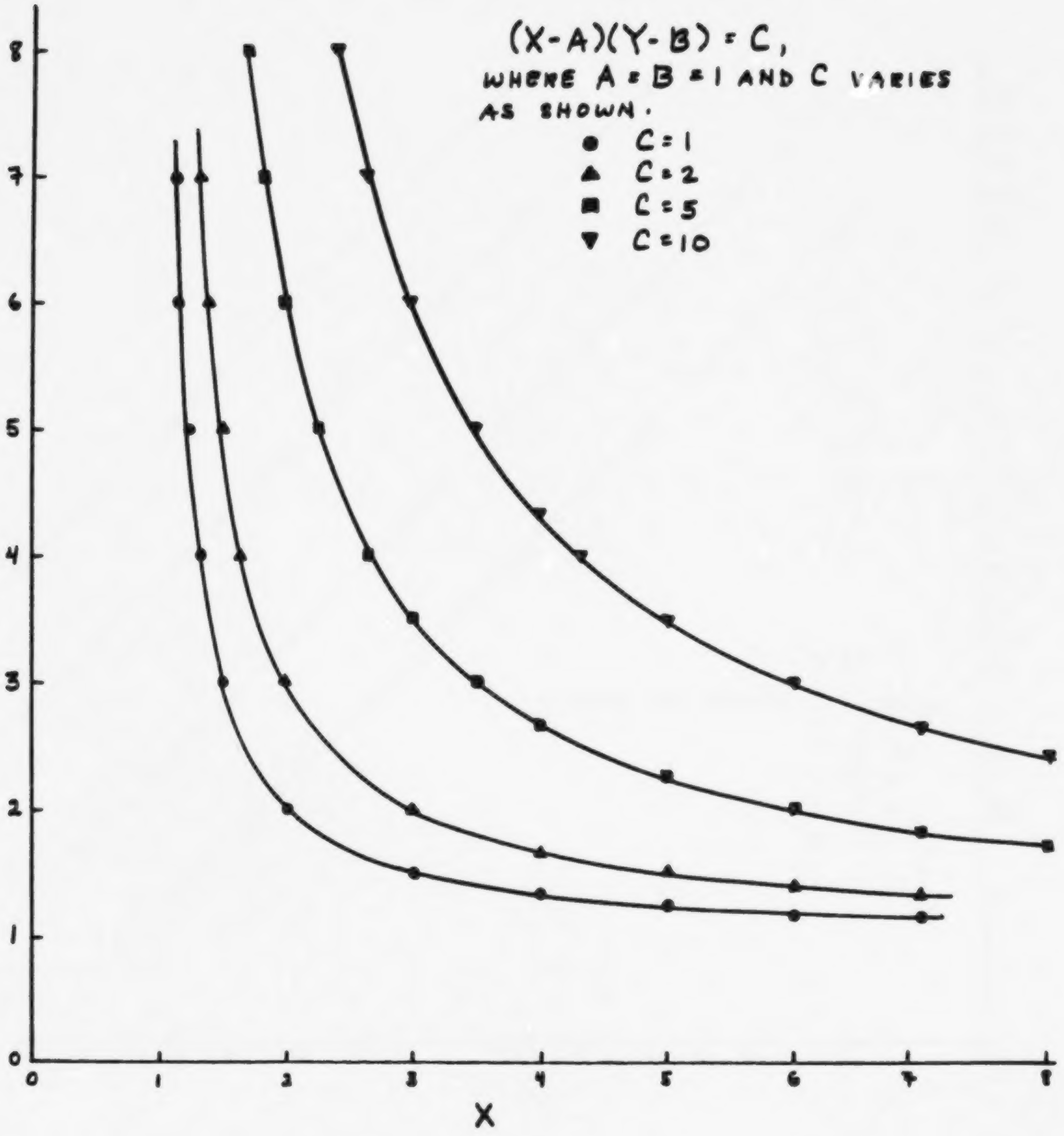


FIGURE 8

Time-weighted dose averaging using an arithmetic coordinate system and incorporating a threshold dose and a minimum time to effect (A and B, both equal to 1 in this illustration). Plotting in an arithmetic coordinate system produces a family of hyperbolas similar to those shown in Figure 6 but shifted in position.

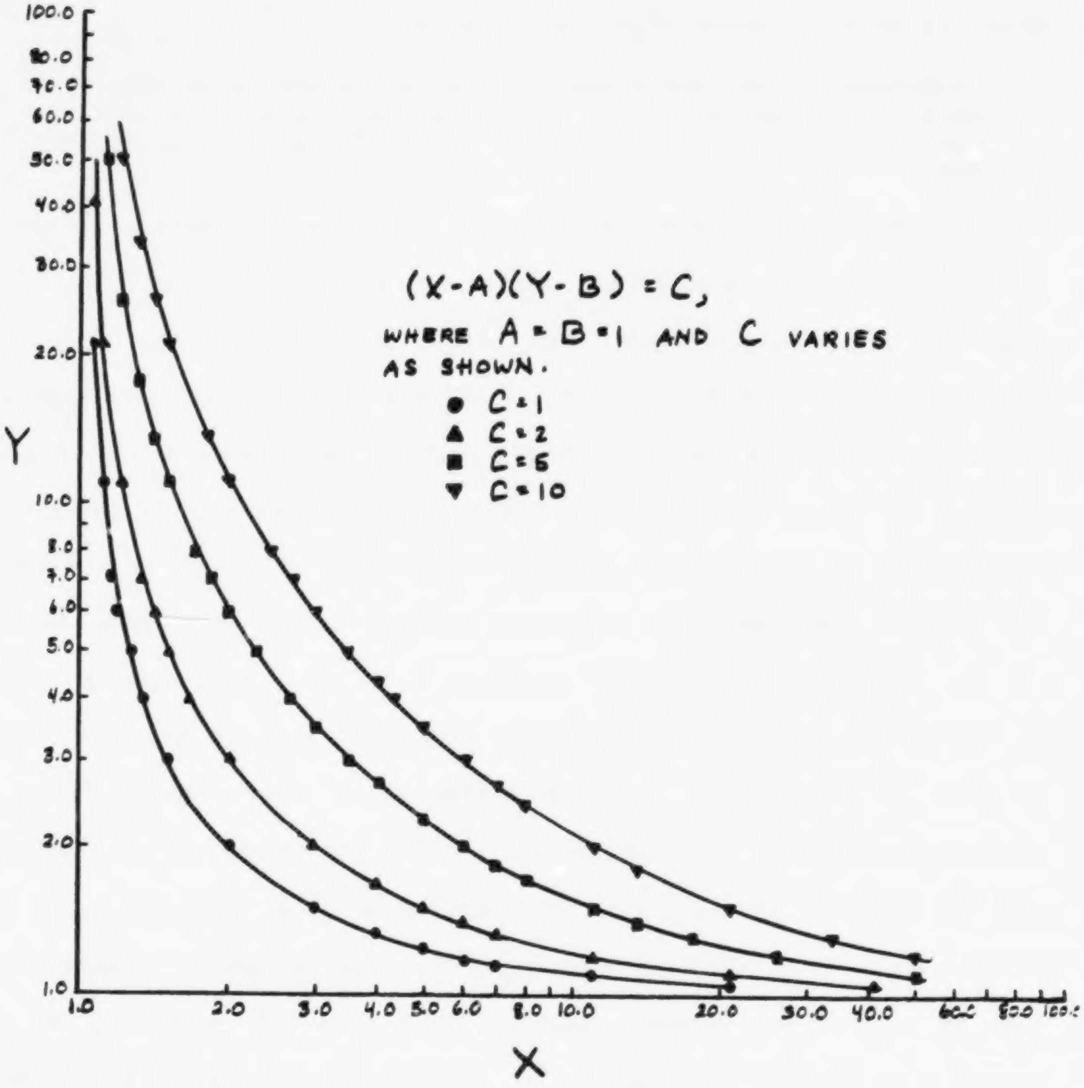


FIGURE 9

Time-weighted dose averaging using a log-log coordinate system and incorporating threshold dose and minimum time to effect. Plotting the data from Figure 8 in a log-log coordinate system does not produce straight lines. Compare with Figure 7.

threshold dose or minimum time is approached, as well as by the chemical or physical nature of the substance under consideration. Filov et al. (1979) appear to recognize something of this problem:

> Furthermore it has been shown that, other factors being equal, the angle of inclination (e.g., the slope) for one and the same substance will be smaller the closer its effect is to the threshold level.

This statement mixes concentrations and effects, but recognizes the practical existence of thresholds and appears to concede that log-log plots of dose rate vs. time-to-occurrence may not be linear throughout. Thus, it is not advisable to plot dose rate vs. time data as a straight-line segment in a log-log coordinate system. This procedure would make it difficult to identify threshold dose and minimum time-to-occurrence, should these exist. Furthermore, if data had been obtained on an "arm" of the curve in Figure 9, the slope assigned would be determined largely by the dose range and would not be characteristic of the compound or of its mechanism of action. Fitting of the equation of a hyperbola with asymptotes not necessarily equal to zero, as in Figure 8, is to be preferred for two reasons: first, the minima (which may be zero) can be calculated; and second, if available data span the midpoint of the curve, then observance of a slope not equal to -1 at this point is meaningful, suggesting that dose-dependent factors (induction of detoxifying enzymes, saturation of metabolic pathways) or time-dependent factors (alterations in host sensitivty) are operating to skew the relationship of dose rate to time.

All these calculations are based on the assumption that the total (integrated) dose presented to the individual is the sole determinant of effect; at exposure times much less than the half-life of the compound of concern, this may be true. In other words, this assumption is reasonable for exposure periods during which processes of metabolism and excretion are relatively less important determinants of body burden than is rate of entry into the body. At exposure times less than the half-life, body burden increases steadily with continuing exposure at a rate roughly proportional to exposure rate. However, at exposure times greater than the half-life, the rate of increase of body burden with continuing exposure declines until eventually the body burden reaches constancy at a steady-state value. The above approaches to calculation of total effective dose would not be appropriate over such long exposure periods.

This is a particularly important consideration when exposure occurs repeatedly (i.e., intermittently). Elimination processes, operating during the intervals between exposures, can have a large impact on total internal exposure. For example, Figure 10 (O'Flaherty, 1981) shows the ratio of body burden after n equal and equally spaced intravenous doses [BB(n)] to body burden after one dose [BB(1)] as a function of $K_e\Delta t$. Clearly, as elimination rate constant k_e is increased with the interval Δt between doses remaining constant, the number of doses required to achieve a stipulated body burden (i.e., that body burden associated with a specified effect) also increases. It is clear that processes of elimination are important determinants of total internal exposure whenever exposure continues for an extended period of time, and that they should be taken into account whenever comparisons between shorter and longer exposures are made.

Conclusions

To obtain data over a limited concentration and time range and then to attempt to linearize them is not justifiable.

The log-log transformation approach is most applicable to chronic exposures to relatively constant concentrations or dose rates. For intermittent exposures, other considerations need to be made. Acute dose data may be

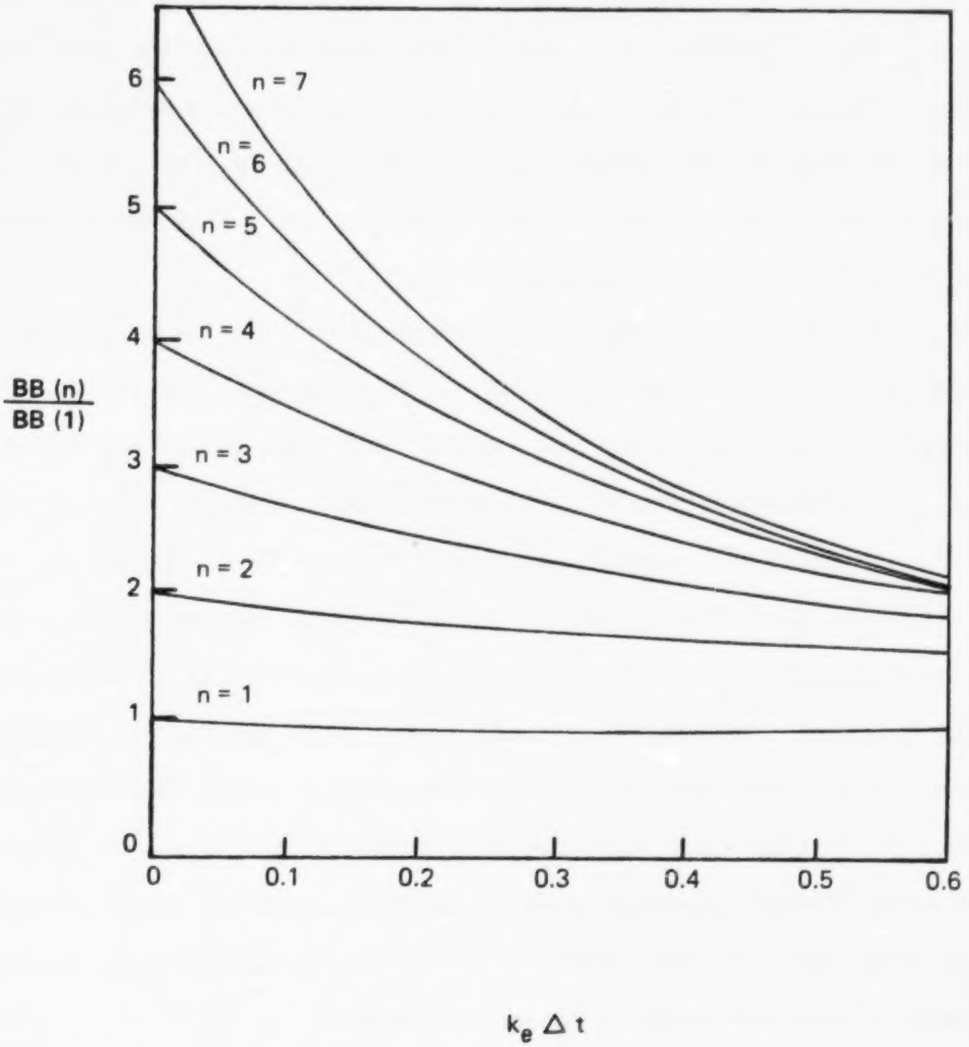


FIGURE 10

The ratio of body burden after n equal and equally spaced doses [BB(n)] to body burden after one dose [BB(1)] as a function of $k_e\Delta t$, for values of n from 1-7, in the one-compartment body model.

useful to the toxicity of the compound if the critical effect appears very early relative to the half-life of the compound. If the critical effect appears later, and exposure is intermittent (as is usually the case), then ideally we should use the following data:

1. Kinetics of the compound
2. Considerations of thresholds
3. Pharmacodynamic considerations (method for dealing with different exposures). It may be that the pharmacodynamic factors (i.e., the concentration at the receptor sites and the relationship between that concentration and effect) rather than pharmacokinetic measurements will be the key determinant of a compound's toxicity.

DISCUSSION

DR. MYRON MEHLMAN

The approach suggested by Rolf Hartung is very interesting. However, I would like to see data or a series of tables on at least several hundred chemicals that can be converted from mg/kg to mg/m² of body surface. Such calculations might be very useful for estimating proper toxic dose levels for different species.

DR. KENNETH CRUMP

I feel that we have not yet arrived at a suitable solution to the dose-duration problem for systemic toxicants. While the dose-duration graphs can be useful in summarizing data, in most cases the data may be too limited to permit full use of this method. Even when the graphs are available, one still has the problem of how to use the graph in setting ADIs. At this point, I have no clear ideas about how to proceed.

MR. WILLIAM GULLEDGE

Given a well-conducted animal study and the consideration of negative data in some manner, the uncertainty of the data base would be kept to a minimum. In this instance, the uncertainty associated with extrapolating laboratory test results to human health effects would be greater. Use of a 10-fold uncertainty factor for each step in the extrapolation process may be inappropriate for universal application. Lower confidence limits associated with the data as well as determination of the LOAEL on a case-specific basis could lower the 10-fold value for several steps. The 10-fold uncertainty factor in comparing animal data to human data appears to be extremely suspect in many cases.

DR. RICHARD KOCIBA

The issue of interspecies conversion was again addressed, with several factors indicating one cannot always use mg/cm² surface area as the proper basis of extrapolation (Reitz et al., 1978). One should extrapolate on the basis indicated by the information regarding whether the metabolic processes lead to activation or detoxification of the material. The comments of Drs. Hartung and O'Flaherty in their presentations should be reviewed in this regard.

The issue of adjusting for differences in duration of experiment and lifespan must be addressed in view of the limitations inherent in any simplistic approach, such as the use of Haber's rule, which appears to be applicable only when dealing with relatively minor differences in dose or duration. For lifetime cancer studies, 24 months should be used as representative of the lifespan of rats, with 18 months for mice.

DR. HARRY SKALSKY

Species-specific metabolism generally presents the most difficult extrapolation problems. However, it appears that *in vitro* techniques are evolving into useful predictive tools. On the following page, note in the table the comparative deaminase activity, used by Dedrick and Bischoff (1980) in estimating human serum concentrations of cytosine arabinoside from animal data (Table 4). It would appear that a compendium of this type of species-specific data would serve as a valuable resource for the Agency, and be well worth the effort of gathering.

GENERAL COMMENTS

- It isn't necessary to only use linear relationships; we can handle non-linear relationships for duration of exposure. For interspecies conversions, introduction of structural parameters may effectively linearize the relationships, increasing the correlation coefficient.

TABLE 4

Model Parameters for Cytosine Arabinoside in Several Species*

Parameter	Mouse	Monkey	Dog	Man
Body weight (g)	22	5000	10,000	70,000
Volume (mℓ)				
Blood	1.67	367	670	2,670
Liver	1.30	135	230	1,700
Gut	1.30	230	400	3,180
Heart	0.095	17	54	450
Kidney	0.34	30	50	1,060
Lean	10.0	2000	4,300	27,000
Marrow	0.60	133	270	2,000
Blood flow (mℓ/min)				
Blood	4.38	431	805	4,040
Liver	1.80	133	270	1,430
Gut	1.50	125	216	1,100
Heart	0.28	63	54	240
Kidney	1.30	123	216	1,240
Lean	0.83	67	223	930
Marrow	0.17	23	40	180
Michaelis constant (µg/mℓ H_2O)	283	39	115	39
Deaminase activity (µg/g-min)				
Blood		1.6		
Liver	4.6	140.2	7	119
Gut	8.3			
Heart		37.0		6
Kidney	91.5	71.8		20
Lean		34.3		
Marrow				
Kidney clearance (mℓ/min)	0.18	14	32	??

*Source: Dedrick and Bischoff, 1980 (Copyright permission granted)

- Guidelines similar to those available for carcinogens should be established for a data base that could be used to develop measures of risk for systemic toxicants.

- Body surface area cannot be categorically assumed to be the only way to make interspecies conversions. The appropriateness of this approach has been looked into by Reitz et al. (1978) who evaluated chloroform in rats and mice on the basis of surface area. They concluded that the mouse data did not accurately predict the effect in rats.

- One of the most effective ways to extrapolate may be blood level concentration.

- Since exposure is usually intermittent, we need some conceptual way of converting from our knowledge of the effect at a constant dose level to the likely effect of intermittent exposure. We need to advance our knowledge by systematic compilation of the available information on the effect of fractionation of doses for relevant toxics.

- Fractions do not always add up to the sum of the total due to such factors as repair and specific repair mechanisms.

- When discussing intermittent vs. long-term exposure, one must take target tissue into account since intermittent exposure to the CNS where cells are not replaced is totally different than exposure to an organ which can be repaired, such as the kidneys.

- When looking at intermittent exposures, the concentration is more important than the total dose. Different endpoints may be affected differently, making generalizations impossible.

REFERENCES

Dedrick, R.L. and K.B. Bischoff. 1980. Species similarities in pharmacokinetics. Fed. Proc. 39: 54-59. [This article based on Dedrick, R.L., D.B. Forrester, J.N. Cannon, S.M. El Dareer and L.B. Mellett. 1973. Pharmacokinetics of 1-β-D-arabinofuranosylcytosine (Ara-C) deamination in several species. Biochem. Pharmacol. 22: 2405-2417.]

Dixon, R.L. 1976. Problems in extrapolating toxicity data for laboratory animals to man. Environ. Health Perspect. 13: 43-50.

Filov, V.A., A.A. Golubev, E.I. Liublina and N.A. Tolokontsev. 1979. Quantitative Toxicology. John Wiley and Sons, New York, based on the 1973 Russian edition of Kolichestvennaya Toksikologiya, translated by V.E. Tatarchenko. p. 50.

Freireich, E.J., E.A. Gehan, D.P. Rall, L.H. Schmidt and H.E. Skipper. 1966. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. Cancer Chemother. Rep. 50: 219-244.

Harwood, P.D. 1963. Therapeutic dosage in small and large mammals. Science. 139: 684-685.

O'Flaherty, E.J. 1981. Toxicants and Drugs: Kinetics and Dynamics. John Wiley and Sons, New York. p. 370-374.

Pinkel, D. 1958. The use of body surface area as a criterion of drug dosage in cancer chemotherapy. Cancer Res. 18: 853-856.

Reitz, R., P.J. Gehring and C. Park. 1978. Carcinogenic risk estimation for chloroform: An alternative to EPA's procedures. Food Cosmet. Toxicol. 16: 511.

Schein, P.S., R.D. Davis, S. Carter, J. Newman, D.R. Schein and D.P. Rall. 1970. The evaluation of anticancer drugs in dogs and monkeys for the prediction of qualitative toxicities in man. Clin. Pharmacol. Therap. 11: 3-40.

Wagner, J.G. 1971. Dosage of drugs in infants, children and adults. In: Biopharmaceutics and Relevant Pharmacokinetics, Drug Intelligence Publications, Hamilton, IL. p. 21.

Weil, C.S. and D.D. McCollister. 1963. Relationship between short- and long-term feeding studies in designing an effective toxicity test. J. Agric. Food Chem. 11: 486-491.

Weil, C.S., M.D. Woodside, J.R. Bernard and C.P. Carpenter. 1969. Relationship between sirgle per oral one-week and 90-day rat feeding studies. Toxicol. Appl. Pharmacol. 14: 426-431.

SYSTEMIC TOXICANTS

Risk Assessment for Less-Than-Lifetime Exposure

Presentation:	Dr. Richard Hertzberg ECAO, OHEA, U.S. EPA
Critique:	Dr. Sheldon Murphy University of Texas at Houston
Critique:	Dr. William Nicholson Mt. Sinai Hospital

PRESENTATION

DR. RICHARD HERTZBERG: RISK ASSESSMENT FOR LESS-THAN-LIFETIME EXPOSURE

Exposure durations which are less-than-lifetime are not clearly defined. Terms used most often are, in order of increasing duration: acute, short-term, subchronic and chronic. These terms have been defined for rodents only. One overriding concern is therefore how to express the duration of an animal study as an equivalent human exposure duration. Percent lifetime has been suggested, but has not yet been supported by actual data. The following sections discuss reasons for considering exposure duration and explore the kinds of data needed for quantitative adjustment of animal data for use in human risk assessment.

Present Approach

There is currently no approach for estimating human health effects from less-than-lifetime exposures and there is no method for estimating a partial lifetime ADI. Although actual studies are conducted and used for determining acute water criteria for aquatic organisms, no corresponding programs exist for estimating acute criteria for humans. The only human health calculation which considers exposure duration divides the subchronic exposure level (usually a NOAEL) by 10 to estimate the corresponding chronic level. The reverse procedure, to estimate a subchronic level, has not been recommended or used.

Possible Approaches

One obvious approach, similar to that used for aquatic organisms, is to examine the toxicity data on a chemical to see if any clear trends are evident which relate dose/exposure to duration for a similar type of effect. For example, Figure 11 shows rough relationships between human equivalent dose (based on mg/day/body surface area) and duration for several categories

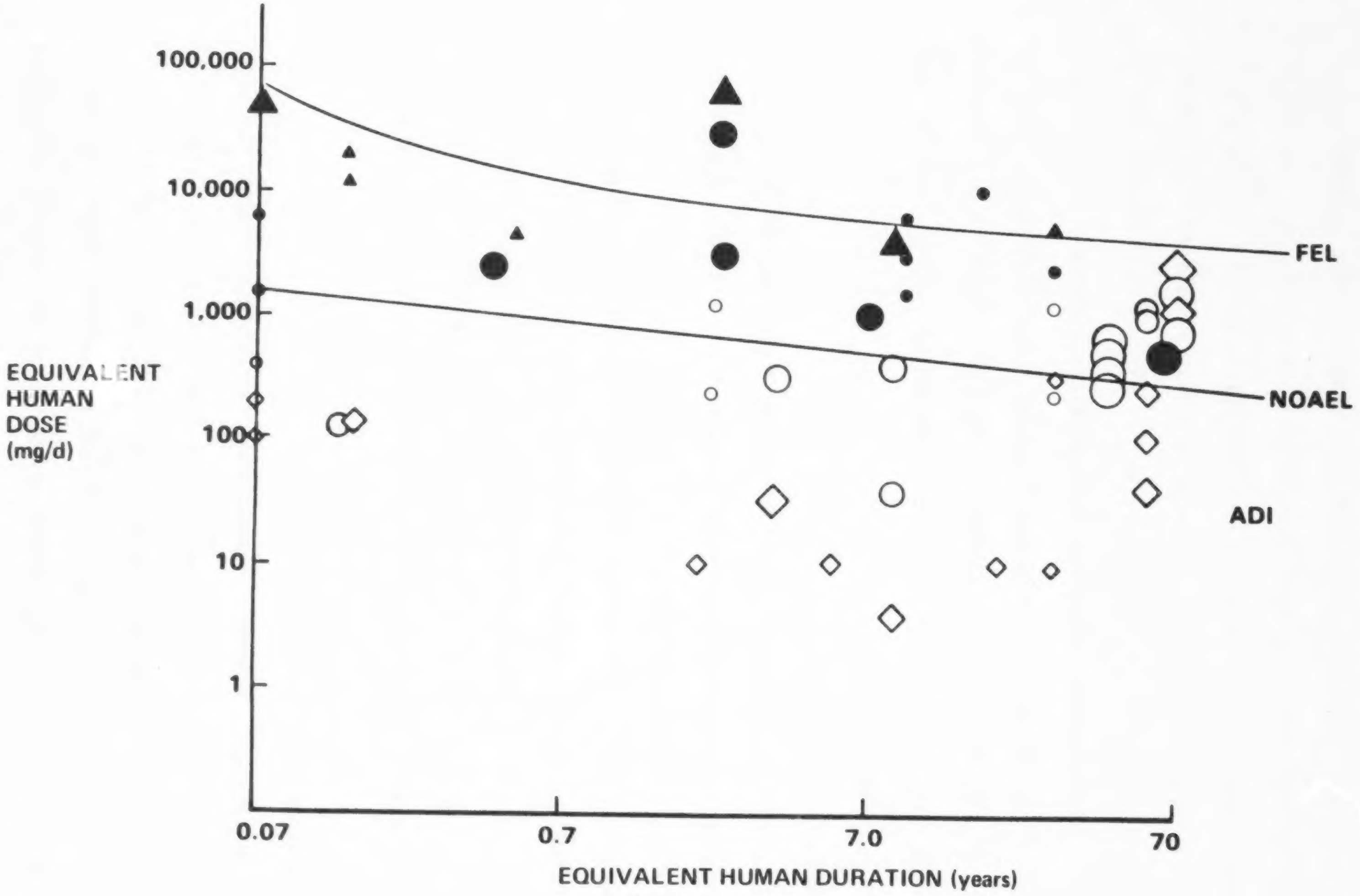


FIGURE 11

Dose-duration Relations Based on Several Toxicity Studies

Effect Level: ▲ = FEL; ● = AEL; ○ = NOAEL; ◇ = NOEL

Study Quality: ● = Good; ● = Average; ● = Poor of health effect severity. No statistical techniques have yet been developed for these trend lines. A major assumption in constructing such graphs is that percent lifetime is a valid index of duration across species. Since each graph summarizes studies by different investigators, further uncertainties exist, including differences in study protocols and the subjective judgment in determining the severity of each effect.

Some journal articles have examined the relationships among doses for different durations. The relationships are empirical rather than mechanistic since they are not necessarily for equitoxic dose rates. For example, Weil et al. (1969) compared LD_{50}s to subchronic minimum effect levels. Thus generalizations to a wider group of chemicals may not be justified. Several reviewers of our methodology have suggested that, in general, subchronic and chronic studies are likely to show similar effects at similar doses, and that an acute acceptable dose might be no more than 10 times the chronic acceptable dose.

Metabolism and pharmacokinetics data would alter the above general approaches. Rapid accumulation to a steady-state tissue or blood level suggests that short-term through lifetime exposures would depend only on daily dose rate, not duration. The use of uptake and excretion half-times should be cautious, however, since a chemical may not have a single half-time, but one which depends on species, dose rate, target organ, other chemicals or conditions (such as plasma pH), etc. This caution also applies to other quantitative information, such as rate of enzyme activation, extent of disposition in tissues and any general adaptation to a constant dose rate. The extent to which such data might alter the eventual risk assessment can be investigated, since tested models exist for multicompartment kinetics, single oral and multiple oral dosing, and enzyme induction. Another factor is the actual disease progression. If effects have a latent period, then short but high exposures may be quite damaging to young people but much less so to the elderly. Until more data become available and tested, the above approaches will most likely remain as only qualitative considerations and not the quantitative modifications we would prefer.

One area which might be quantifiable is exposure during a critical period of fetal development. If these "windows" of time can be identified for experimental species and humans, then short-term or acute exposures to mothers could be better evaluated.

CRITIQUES

DR. SHELDON MURPHY

ADI may be estimated without lifetime exposure. MacNamara (1976) showed that data derived from studies at less than 90-day exposure can be useful. With 95% confidence, a dosage which would produce no effects during a lifetime of exposure could be predicted from the 3-month, no-effect dose with the application of a safety factor or uncertainty factor of 10. MacNamara's conclusion was that it is possible to predict a NOEL in probably all cases except carcinogenesis and reproductive effects. He also proposed shortened versions of how to obtain sufficient data to make assessments of these long-term effects.

Spyker (1975) summarized effects resulting from exposure of embryos to mercury *in utero*. Behavioral deviations were only observed in maturing animals. Neurological disorders were only apparent at maturity. Early senescence was only apparent late in life; premature death was only apparent at death. He concluded that a shorter duration study may not have detected effects, since these effects only occurred at certain stages of lifetime.

If you have to observe over a significant period of the lifetime to see effects, is there a cost advantage to limiting exposure to 90 days?

In terms of risk assessment and predictability of interactions, if we know enough about mechanisms, we may reduce risk assessment to an integrated series of *in vitro* and short *in vivo* tests.

Conclusions

1. With the possible exception of carcinogenesis and reproductive effects, it appears that a good 90-day (or short-term) study will predict qualitatively, and perhaps, in most cases, quantitatively long-term effects.

2. Quite possibly the period in life in which a short-term exposure occurs will influence the type as well as the dose required for effects over a lifetime observation period.
3. If the objective of short-term tests is to reduce the cost of testing, will this be accomplished if long-term or lifetime observations are still required?
4. With regard to multiple chemical exposures, it seems that the objective should be to determine how the presence of one or more additional chemicals should influence the hazard assessment or the ADI of individual chemicals, not to develop ADIs for mixtures.
5. If we know mechanisms and affinity constants and rate constants for interactions of chemicals with critical macromolecules, we should, through appropriate mathematic models, be able to make predictions of possibly interactive situations.

DR. WILLIAM NICHOLSON

Use of a factor of 10 for extrapolating short-term to chronic toxicity would appear to be reasonable based on the ADI data of Weil and McCollister (1963) in which most subchronic to chronic effect ratios were less than 6; only 5% exceeded the value of 10. Because of the possibility that there could be significant differences between chronic and subchronic ADIs, I would suggest that a factor no less than 10 be utilized. Just to emphasize an obvious point, the inverse extrapolation from chronic to subchronic should utilize no alteration in the level. While the subchronic and acute time periods are somewhat ill defined in the context of the extrapolations to lifetime exposures, I would suggest that a factor of 10 be applied to subchronic exposure circumstances that are no less than 10% of the lifetime of the experimental animal.

In certain cases, for example lung cancer in asbestos workers, age at exposure has a bearing on the health impacts. For older people, the effects of asbestos exposure can be more serious in shorter periods of time than would be predicted by the model for mesothelioma. People exposed at a young age might not contract lung cancer until age 50, but if an older person, already at high risk from cigarette smoking, is exposed at age 45 or so, lung cancer can appear in 5-10 years. In the case of angiosarcoma, older animals are more susceptible.

An understanding of the mechanism of action is necessary before applying mathematical models. Short-term effects seen in animals may not appear after chronic exposure. Human chronic effects may not be observed in animals. Multiplicative safety factors are needed and should continue to be used until we have a sufficient data base to use other models.

DISCUSSION

DR. MYRON MEHLMAN

It is now clear that subchronic studies (90-180 days) have a high degree of predictability for chronic systemic toxicity studies and could be utilized to assess injury from long-term exposure. In addition, based on recent data, carcinogenicity may possibly be predicted from early subchronic studies. This requires extensive pathology experience.

DR. MAGNUS PISCATOR

The influence of plasma pH on half-time is probably insignificant. Even in conditions of acidosis and alkalosis, the plasma pH shows small changes. I suggest that "acid-base status" is used instead.

DR. RICHARD KOCIBA

My comments on this are reflected in the previous chapter (Interspecies Conversion of Dose and Duration of Exposure). Overall, the empirical basis for relating acute to subchronic, and subchronic to chronic durations via use of appropriate uncertainty factors has been found to be a useful tool and should be continued.

GENERAL COMMENTS

- In connection with MacNamara's data (MacNamara, 1976), there is no trend that points out pharmacokinetic relationships of the ratio of LD_{50}/lifetime exposure. Biological effects need to be considered as well as the concentration.
- High doses for a short period may not equal low doses for a long period. Progressive increases in body burden would result from bone-seeking compounds and some lipid seekers.
- For unknown compounds, a battery of tests that produce no false negatives is needed.
- We must have data to estimate the risk of false negatives.
- Short-term tests should be used as indicators for doing long-term testing. Decisions regarding chronic toxicity should be based on chronic tests, not short-term tests.

- Testing should deal with the problem of estimating human risk from intermittent exposure.

- Occupational exposure data could be used to estimate human exposure.

REFERENCES

MacNamara, B.P. 1976. Concepts in health evaluation of commercial and industrial chemicals, Chapter 4. In: New Concepts in Safety Evaluation, M.A. Mehlman, R.E. Shapiro and H. Blumenthal, Ed. Hemisphere Publishing Corporation, Washington, DC. 455 p.

Spyker, J.M. 1975. Assessing the impact of low-level chemicals on development: Behavioral and latent effects. Fed. Proc. Fed. Am. Soc. Exp. Biol. 34: 1835-1844.

Weil, C.S. and D.D. McCollister. 1963. Relationship between short- and long-term feeding studies in designing an effective toxicity test. J. Agric. Food Chem. 11: 486-491.

Weil, C.S., M.D. Woodside, J.R. Bernard and C.P. Carpenter. 1969. Relationship between single per oral one-week and 90-day rat feeding studies. Toxicol. Appl. Pharmacol. 14: 426-431.

SYSTEMIC TOXICANTS

Incidence and/or Severity of Effects

Presentation:	Dr. Kenneth Crump Science Research Systems
Critique:	Dr. Robert Neal Chemical Industrial Institute of Toxicology
Critique:	Dr. Ronald Wyzga Electric Power Research Institute

PRESENTATION

DR. KENNETH CRUMP: HOW TO UTILIZE INCIDENCE AND/OR SEVERITY-OF-EFFECT DATA IN SETTING ALLOWABLE EXPOSURES

Subissues

1. How to account for severity of effects (acute lethality, cancer, weight loss, changes in blood pressure or plasma enzyme levels, etc.).
2. How to utilize different types of data including: incidence data (number of animals dead or with tumors, etc.); "continuous" data (average levels with standard errors, etc.); limited or graded data (severe, moderate or no liver necrosis, etc.).

Possible Options

1. (Used previously to set water quality criteria.) If carcinogenic, extrapolate using linearized multistage model. If not, use the safety factor approach (apply a safety factor to a NOEL, NOAEL or LOAEL).

Pro: Minimal data requirements.

Has been tested and is familiar to most.

Relatively simple to apply.

Con: Safety factor approach doesn't fully utilize shape of dose-response curve.

With safety factor approach, smaller studies tend to yield higher allowable exposures, which is illogical.

Choice for safety factors is largely judgmental.

Inconsistencies may arise from applying different methods to cancer and non-cancer data.

2. Extrapolate both incidence and continuous data to low doses using mathematical models. Continuous data could be extrapolated to a dose corresponding to a certain percent change in normal levels or a certain fraction of the standard deviation within a normal population. Extrapolation to different levels could account for differing severity of

disease (e.g., extrapolate cancer data to 10^{-5} lifetime risk and weight loss data to 10^{-2}). The smallest allowable exposure obtained from any given health effect could be selected as the standard.

Pro: Accounts for shape of dose-response curve and utilizes all the experimental data.

"Rewards" larger experiments and those with better experimental designs (if confidence intervals are used).

More objective than safety factor approach.

Is not strongly dependent upon choice for mathematical model.

Con: Choice of extrapolation model is judgmental.

Has greater data requirements than Option 1.

Marginally more costly to implement than Option 1.

3. Use mathematical models to estimate dose corresponding to 10^{-1} or some other level in the "observable range", and apply a safety factor reflecting the serverity of the health impairment and possibly the nature and extent of the data.

Pro: Accounts for shape of dose-response curve and utilizes all the experimental data.

"Rewards" larger experiments and those with better experimental designs (if confidence intervals are used).

More objective than safety factor approach.

Is not strongly dependent upon choice for mathematical model.

Con: Choice for safety factor is large judgmental.

Has greater data requirements than Option 1.

Marginally more costly to implement than Option 1.

CRITIQUES

DR. ROBERT NEAL

In Dr. Crump's presentation, he indicated he would not disucss extrapolation from animal data to man. However, in my opinion the entire purpose for setting allowable exposures is to protect man. Therefore, my comments on Dr. Crump's presentation will assume that the primary purpose for setting allowable exposure levels is to protect man and will therefore include considerations of extrapolation from animal data to man.

Dr. Crump has proposed three options for setting allowable exposures to toxic chemicals. Option 1. For cancer-causing compounds he proposed the use of linearized multistage models for extrapolating data obtained in the observable range using experimental animals to low levels of exposure experienced by man. Alternatively, if the compound is not carcinogenic, a safety factor approach should be used, i.e., a safety factor applied to the NOEL, NOAEL or LOAEL.

Option 2. Alternatively, he proposed that the risk estimation for both carcinogenic and noncarcinogenic compounds could be carried out using mathematical models to extrapolate to different levels of risk depending upon the severity of the disease (i.e., more risk for cancer, less risk for weight loss).

Option 3. Finally, he proposes using mathematical models to estimate the dose which provides a calculated incidence level for both carcinogenic or noncarcinogenic effects (e.g., 10% or 1% incidence) and then applying a safety factor to the calculated dose. In this case the safety factor could vary depending on the severity of the chemically-induced disease.

It is my opinion that the use of safety factors applied to a NOAEL for toxic effects other than cancer is the most appropriate approach. This approach has been used for a number of years in regulating human exposure to potentially toxic compounds. The data to date suggest that this approach has served us well in that there is no evidence of substantial adverse human health effects from exposure to regulated levels of compounds determined using these procedures. The most appropriate safety factor to use in determining the allowable exposure level should range from 10-100 depending upon the severity of the effect.

In a report of their Safe Drinking Water Committee, the National Research Council (NRC, 1977) has proposed that a 1000-fold safety factor be applied to compounds for which the data are incomplete. In my view, the lack of data does not warrant this more conservative safety factor. The choice of an appropriate safety factor should be dictated by the degree to which we understand 1) the mechanism of the observed toxic effect and 2) the applicability to man of the data generated in experimental animals. In those cases where we do understand the mechanism of toxicity and applicability of the animal data to man, a smaller safety factor should be used. For example, we understand the mechanism of acetylcholinesterase inhibition by organophosphate and carbamate insecticides to a reasonable degree. We also have knowledge of the applicability to man of quantitative acetylcholinesterase inhibition data generated in rats and mice to man. Therefore, a safety factor of 10 is often applied to the NOEL for cholinesterase inhibition in rats and mice in calculating the allowable exposure to man to compounds inhibiting this enzyme. Thus for compounds where the mechanism of toxicity is known and the applicability of the data from experimental animals to man is also known, a safety factor less than 100 should be considered. In cases where these factors are not well understood, a more conservative safety factor should be applied, particularly when the adverse health effects are essentially irreversible (cancer, neurotoxicity, teratogenicity, reproductive toxicity).

The key missing element for extrapolating is a sufficient data base for dose/response, namely, the raw data on the numbers of animals that have certain effects. Without this we lack confidence in using mathematical models for extrapolating to low incidence rates. We can use a lower safety factor if we know the toxicity mechanism and have confidence in the predictability of our animal models. We can use a higher safety factor if we don't know the toxicity mechanism and don't have confidence in the predictability of animal models.

DR. RONALD WYZGA

I agree with Dr. Crump's general description of the pros and cons of the three options. I would add or emphasize an additional con for the first option -- the NOAEL-plus-safety-factor approach. The result is dependent upon the number and kind of dose levels used in the available experiments. If, for example, experiments were carried out at only high dose levels, the criteria could differ significantly from experiments in which only low dose levels were used. Hence under Option 1, point X_2 in Figure 12 could be defined as a NOAEL and a safety factor could be applied. If, however, the dose corresponding to X_2 were not included in the experiment, the NOAEL would be X_3, thus demonstrating the dependence of NOAELs upon the dose levels of the underlying experiments.

I agree with Dr. Crump's assessment of Option 2, but would emphasize that we'll never be able to discriminate among models at dose levels corresponding to low risk, and any choice of a model remains arbitrary and potentially controversial, particularly when we are dealing with noncarcinogens.

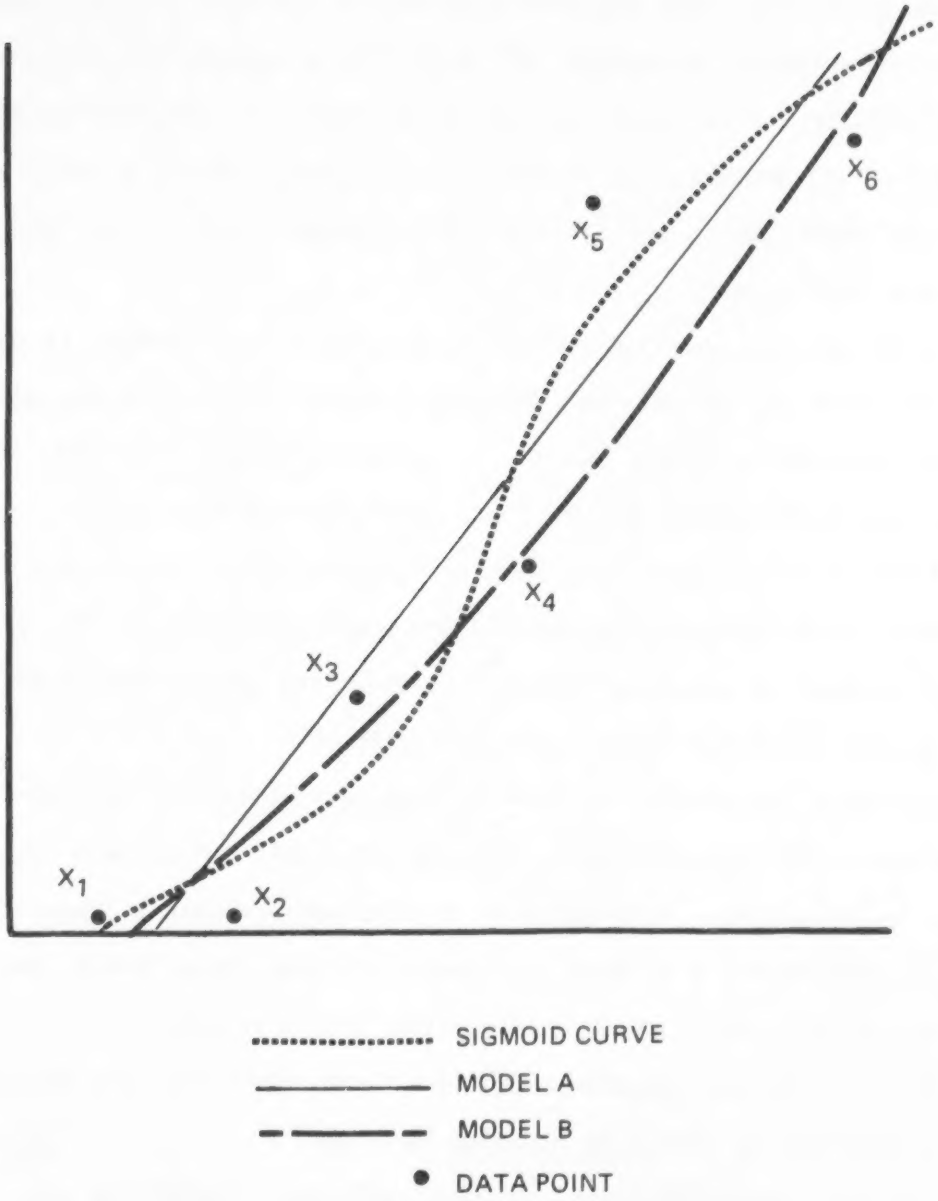


FIGURE 12

Alternative Dose-Response Curves

In the latter case, many different physiological mechanisms are involved, and we don't understand the underlying models for most of them. Moreover, different mathematical models are most likely appropriate for different toxic effects; hence there can be no justification for choosing the same model for all impacts. In Figure 12 for example, Models A and B fit the data reasonably well, yet the differences between them at low dose levels are very significant.

In my opinion, the third option is preferable. It attempts to standardize the first by setting the "NOAEL-equivalent" to the 10% response level before applying a safety factor. I believe, however, that this approach needs some modifications for the four reasons given below.

First of all, I have a problem with the exclusive use of one model to estimate "dose corresponding to 10^{-1} or some other level in the observable range" because of the great uncertainty about the appropriate model to use, and because models can differ considerably at 10^{-1}.

Secondly, the choice of 10^{-1} is somewhat arbitrary. If there are no data near that response level, the use of models to estimate that point could be misleading. Alternatively if data were present at lower response levels, one might have more confidence at lower dose levels than those corresponding to 10^{-1}, and this information should be used.

Thirdly, the use of safety factors remains arbitrary, and arbitrariness could therefore be reduced by avoiding their use.

Finally, the method needs to have sufficient flexibility when supplementary information exists, such as evidence of a threshold, contradictory observational (human) data, recovery mechanisms, and detailed pharmacokinetics.

I'd like to suggest a modification which might be explored as a way to overcome some of the above points. First of all, rather than use 10^{-1} as a point of departure before extrapolation, I suggest one use the lowest response point at which major models converge or the lowest observed point as the departure point. Hence if there is significant variation among models at levels greater than 10^{-1}, the higher level of convergence should be used as the departure point, thereby eliminating uncertainties involved in choosing which model to use to reach 10^{-1}. On the other hand, if model agreement goes below 10^{-1}, one can take advantage of this fact. From the prior ECAO workshop held on February 5, 1982 in Washington, DC, we have seen that model agreement can occur at various points including levels below 10^{-1} and levels much higher than 10^{-1}. This proposed approach considers the consistency of the behavior of a data set with regard to the model structures. If the behavior is consistent, we can make greater use of the models. If the behavior is inconsistent, we then use the lowest non-zero response observation as the departure point.

I would then extrapolate downwards from the departure point to zero. This is less arbitrary a procedure than using safety factors, particularly when the point of departure can vary. (If, however, the point of departure were uniform, a safety factor could be defined which is equivalent to a linear extrapolation.) The linear extrapolation is initially conservative and protective because it can be thought of as an upper bound to the class of sigmoid curves that usually fit biological data. In the data range treated here, these curves are concave and thus bounded by a straight line. The straight-line extrapolation is also consistent with the approach used for carcinogens, since the upper 95% confidence interval of the multistage model, as formulated by Dr. Crump, is essentially a linear extrapolation to zero.

In the absence of further information, one could then determine the criteria associated with a particular risk level, e.g., 10^{-5} or 10^{-6}. I think it is important, however, that the method be sufficiently flexible to incorporate new or additional information when available, and I suggest that the estimated curve be adjusted when warranted.

For example, if one believed or had evidence that a threshold existed for a toxic substance, one could replace the zero at the lower end of the extrapolation by the threshold point or its lower confidence interval. One could do likewise at those levels where human observational data contradicted the animal model inferences. Other mechanisms could be found for treating recovery mechanisms or pharmacokinetics.

The proposed modification offers several advantages:

- It accounts for the shape of the dose-response curve to the extent that the behavior of the available data is consistent with the curve(s).
- It accounts for model variability: when models are consistent with the data, they determine the point of departure; if there is wide disagreement among models, then no one model is anointed without evidence.
- It uses all the data in trying to find the best convergence point for the models.
- It "rewards" larger and better experiments by obtaining better model fits or more data that show the results are inconsistent with model structure. It also can yield a lower departure point when more data are present.
- It is more objective than methods that use safety factors.
- The procedure is conservative when information is scanty, yet it is flexible when additional information is available.
- Because it is flexible, it provides incentives to develop more information, which can lead to better decisions.

The proposed method does, however, have some drawbacks, and these need to be addressed in further detail.

First, because it is a new approach, several details of implementation need to be worked out:

- What models should be considered for convergence? These should be the best candidates that presently would be considered under Options 2 and 3. We probably need more work here in examining alternative models for various sets of data for noncarcinogens.
- What should be the criteria for model convergence? One could consider the overlap of some small confidence interval, such as the 50% level for the models, or require that they will be within a factor of two or so of each other. Again, more thinking needs to be applied to this area; however, it clearly is an obstacle which can be overcome.
- Criteria for adjustment need be established. Again reflection about potential adjustments could lead to a reasonable set of criteria.
- Adjustment procedures need to be worked out. They need to be tailored to the purpose of the adjustment, but can in principle be designed. See, for example, the suggestions given above for threshold behavior and human data.

The proposed method also has greater data requirements than Option 1, but it also makes use of more data and information when they are available. The method is more costly to implement than the other options, but the implementation costs are miniscule compared to the costs associated with the decisions that need to be made.

The proposed method may be too complex. This concern can probably best be addressed only after attempts have been made to apply it.

DISCUSSION

DR. WILLIAM NICHOLSON

Safety Factors for Human Exposures

The use of safety factors as discussed in the documents supplied to us appears quite appropriate. I particularly would support Option 3 proposed by Dr. Crump in which the full set of data is utilized to estimate a risk of 10^{-2} for animals, and then safety factors relating to animal-to-human extrapolation, sensitivity, and short-term vs. long-term exposure are utilized. I would suggest that the terms NOEL, LOEL, etc. in Figure 1 are somewhat misleading. If one defines a level of 1% for the LOEL that level also is the maximum NOEL. Figure 13 provides slightly revised definitions of these terms.

I have a strong reservation about what constitutes a NOAEL. In Figure 1 it was suggested that a slight body weight decrease may be considered such a non-adverse effect. While this may appear true for animals, it may not be so at all for human beings. For example, nutritional defects at an early age leading to slight weight losses have also been shown to significantly affect mental performance, and thus what cannot be measured in an animal (e.g., mental performance -- IQ, if you wish -- behavioral performance reflecting the integrated capacity of the nervous system, etc.) is extremely important for humans. Thus, the application of the safety factors discussed should be applied only to NOELs and not to NOAELs. If the latter are utilized, an additional safety factor, taking into account the non-comparability of measured animal functions with human functions, should be applied. Consider what might have been the consequences had thalidomide led to a decrease of 20 units in an IQ test and not to the obvious birth defects seen.

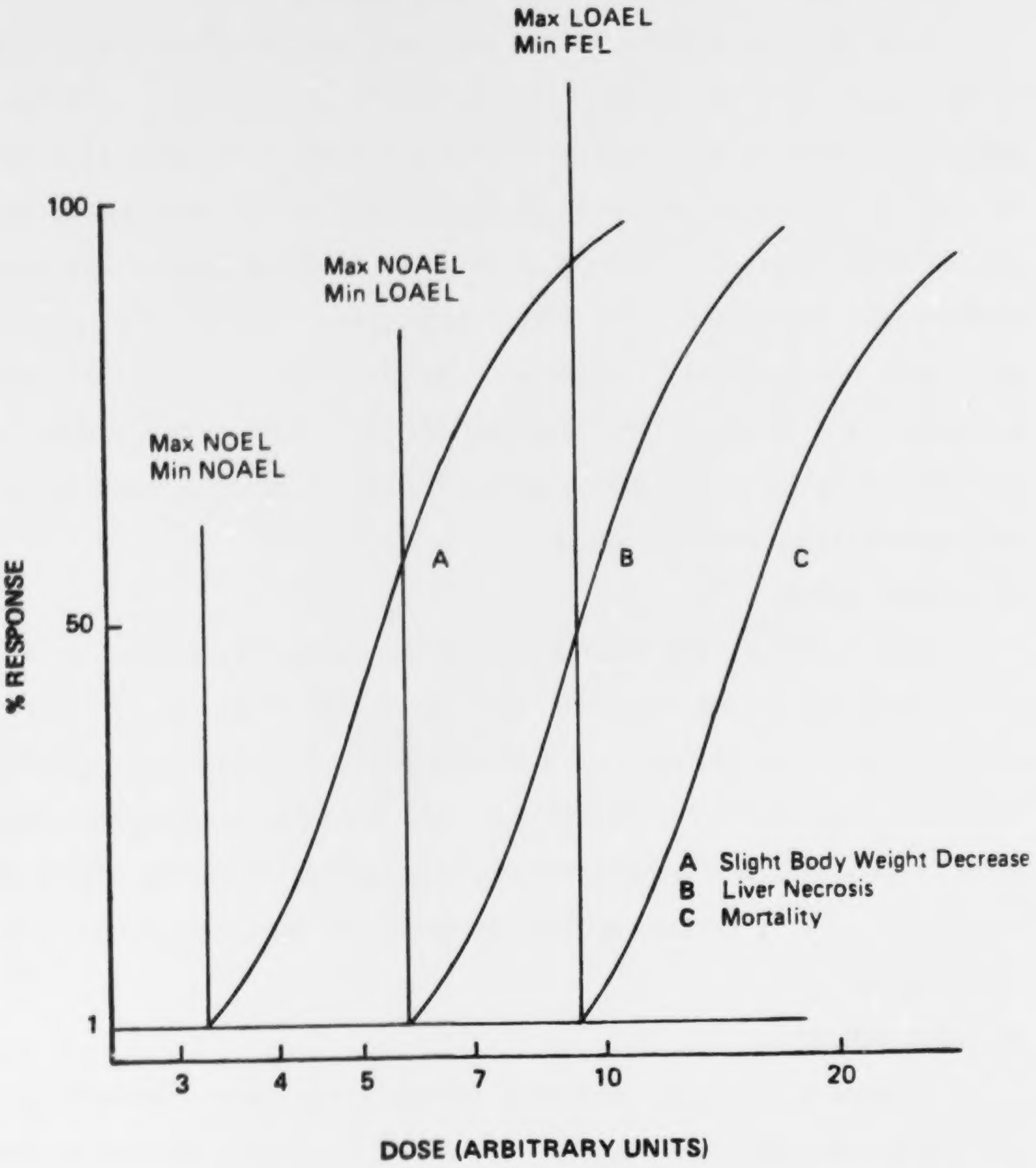


FIGURE 13

Response levels considered in defining threshold regions in toxicity experiments.

DR. SHELDON MURPHY

I think that the question of precisely what extrapolations can or should be made will never be answered to everyone's satisfaction. I liked the approach proposed by Dr. Crump to extrapolate doses to estimate to a 10% or 1% risk of an effect and then apply a safety factor that appropriately considers the nature and severity of effect. As was pointed out by several speakers and discussants, the NOEL/safety factor approach has served us quite well for individual substances. For the most part, harmful chemical exposures have resulted from insufficient toxicological data rather than from failure to apply appropriate safety factors (a possible exception being male antifertility effect of DBCP).

DR. RICHARD KOCIBA

I endorse the present NOEL/safety factor approach as opposed to mathematical modeling of the incidence data and graded data. In most toxicity studies, the histopathology data are the most sensitive parameters defining the NOEL. However, the histopathology data described in literature references is generally more qualitative than continuous. Thus, any mathematical modeling of this is not appropriate and would introduce unnecessary complicating factors.

DR. HARRY SKALSKY

Dr. Kociba's comments concerning histopathology were extremely pertinent. His point was that the NOEL or NOAEL is frequently defined by pathological parameters. These parameters are qualitative judgments and cannot be quantified. This fact supports the traditional safety factor approach and indicates that the "mathematical" model approach would unnecessarily complicate the issue.

DR. IAN NISBET

I support in principle the use of the procedure, proposed by Dr. Crump, involving the use of a model to extrapolate down to the 1% risk level (equivalent to a standardized LOEL), followed by application of safety factors of 100, 1000 or more. As was pointed out, this is operationally equivalent to the use of a low-dose linear model to predict doses associated with risk levels of 10^{-4}, 10^{-5} and lower. Hence, this procedure would have the important advantage of blurring or eliminating the distinction between threshold and nonthreshold effects.

GENERAL COMMENTS

- Model fitting to define a "take-off point" may be advantageous to the regulatory process.
- The model needs to take into account different kinds of data -- dichotomous or graded.
- There is no reason to convert to incidence data; no reason why the model can't be applied directly to graded data. Dr. Wyzga's approach is an "interesting extension" of Option 3, equivalent to taking a multistage approach which has been used for carcinogens. However, this approach might lead to inordinately low levels.
- Histopathology will be the limiting factor, not the graded data. There may be a problem applying a mathematical model to qualitative histopathologic data. Although it is not impossible to quantify histopathologic data, detailed histopathologic data is usually not be accepted by journals.
- Several people have stated that safety factors have served us well and the approach shouldn't be changed. This may not be true. There may have been effects for which we may not have seen the association in the human population.
- Dose-response models have not been proven to hold across species or for extrapolating within species to lower rates.

REFERENCES

NRC (National Research Council. 1977. Drinking Water and Health. Vol. 1. Safe Drinking Water Committee, NAS, Washington, DC. 939 p.

U.S. EPA. 1982. Examination of Options for Calculating Daily Intake Levels (DILs). Prepared by Dr. Kenneth Crump under EPA P.O. No. C2171NAST. January 2, 1982.

Weil, C.S. and D.D. McCollister. 1963. Relationship between short- and long-term feeding studies in designing an effective toxicity test. J. Agric. Food Chem. 11: 486-491.

SYSTEMIC TOXICANTS

Route-to-Route Extrapolation and the Pharmacokinetic Approach

Presentation:	Dr. James Withey Food Directorate, Bureau of Chemical Safety
Critique:	Dr. Ellen O'Flaherty University of Cincinnati
Critique:	Dr. Julian Andelman University of Pittsburgh

PRESENTATION

DR. JAMES WITHEY: ROUTE-TO-ROUTE EXTRAPOLATION AND THE PHARMACOKINETIC APPROACH

In deriving the criteria for ambient water quality, the U.S. EPA noted that, for some substances, no data were available in which the appropriate oral or intragastric route of administration was used (Federal Register, 1979). In these cases, data from inhalation studies or the American Conference of Governmental and Industrial Hygienists (ACGIH) threshold limit values (TLVs) were used in the manner suggested by Stokinger and Woodward (1958). As an example of the application of this principle, the derivation of the ambient water criteria for barium was obtained from the value for its TLV (0.5 mg/m^3) in the following manner.

Inhalation

TLV = 0.5 mg/m^3
Volume inhaled = 10 m^3 (per 8-hour day)
Total amount inhaled = 10 x 0.5 mg/day
Absorption factor (inhalation) = 0.75
Amount reaching systemic circulation = 10 x 0.5 x 0.75 = 3.75 mg

Equivalent Oral Intake

Maximum daily water intake = 2.00 ℓ
Absorption factor (drinking) = 0.9
$\frac{3.75 \times 1}{0.9}$ or 4.17 mg/day may be consumed
4.17 mg in 2.9 ℓ of water is 2 ppm

Limitations of the Stokinger-Woodward method include the following: 1) precisely measured absorption factors are lacking; 2) the TLV may not be based on systemic toxicity; 3) extensive hepatic metabolism (detoxification) may reduce the systemic toxicity by the oral route (first-pass effect) and reduce the toxicological insult compared to an equivalent dose given by another route; and 4) temporal relationships of blood levels, post-administration, are not considered.

Pharmacokinetic considerations allow some judgment as to the validity of assuming equivalent insult for the same dose administered by different routes. An excellent example of an almost identical effect arising from the administration of valproic acid by the oral and i.v. routes in six different subjects has recently been published (Perucca et al., 1978). The similarity of blood-level/time curves following the administration of vinyl chloride monomer in aqueous solution by the intragastric or intravenous routes has also been pointed out (Withey, 1976).

Temporal relationships of blood concentrations during and following the administration of a dose are useful in the assessment of both the magnitude and duration of effect.

Vapors or gases are usually absorbed into the systemic circulation at a constant rate, described by a zero-order process, since the atmospheric concentration of the toxicant remains constant throughout the exposure. Substances administered orally or intragastrically are absorbed by a first-order process, although the uptake mechanism(s) and interactions with other substances within the gastrointestinal tract may be very complex (Barr, 1968). Elimination, which includes metabolism, will usually involve a series of simultaneous and consecutive first-order processes which may be described by multicompartment mathematical models.

It is instructive to compare the concentration of a toxicant in the central pharmacokinetic compartment after dosing by different routes. In this presentation, the effects of a 10-hour vapor phase exposure will be compared to two oral exposures given as four equal divided doses over 10 hours (dose interval = 2.5 hour) and 20 equal divided doses over 10 hours (dose interval = 0.5 hour). In all three exposures, the total administered dose is equal and absorption is assumed to be 100%. For oral exposures, the elimination rate is assumed to be 10 times slower than the absorption rate.

In the simplest case, if a zero-order uptake is assumed for the 10-hour vapor phase exposure and first-order uptake and elimination for one-compartment model after oral dosing, the magnitude of the steady state concentrations on repeated dosing will depend upon the magnitude of the dose and the dose interval (Withey, 1983). Figures 14, 15, 16 and 17 show how blood concentration varies with different routes, dosing intervals and the kinetic parameters for absorption and elimination. These parameters are given in Table 5.

In the first case (Figure 14), vapor phase exposure results in a rapid achievement of steady state (~30 minutes) and a rapid return to zero levels after termination of the exposure (~90 minutes). The four oral doses yield rapid excursions to high peak concentrations (28 µg/mℓ) and a return to zero levels after administration of each dose. The twenty-dose series gives steady state levels with maxima and minima oscillating about the steady state level for the inhalation dose. Clearly, in the case of the four-dose regimen there could be situations in which thresholds would be exceeded which might elicit different toxic effects than those seen with the other protocols. There is no evidence of bioaccumulation if these protocols are extended over several days.

Figure 15 (Case 2) shows blood concentrations that occur when the elimination rate is reduced to about one-tenth of that in Case 1, and the oral absorption rate is kept at 10 times that of the excretion rate. It is evident that steady state conditions do not exist at any time during the exposures and that the twenty-dose regimen very closely approximates the vapor phase exposure. Again, there is no suggestion of a potential for bioaccumulation.

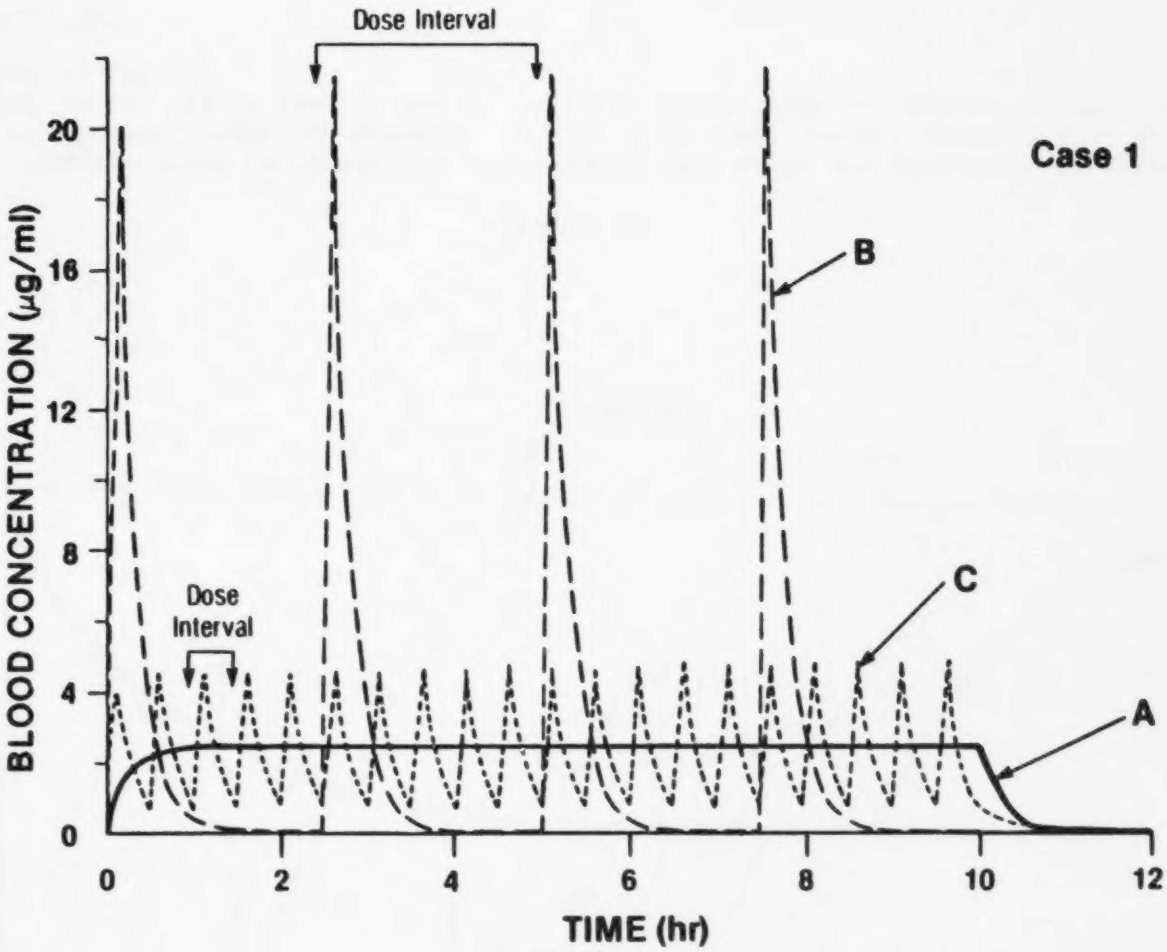


FIGURE 14

Case 1. Temporal blood concentration relationships for uptake by inhalation and gastrointestinal routes. (——) 10-hour inhalation exposure. (— —) four equal divided doses, orally over 10 hours. (----) 20 equal doses, orally over 10 hours. The rate coefficients for absorption and elimination for Case 1 are given in Table 5.

Source: Withey, 1983

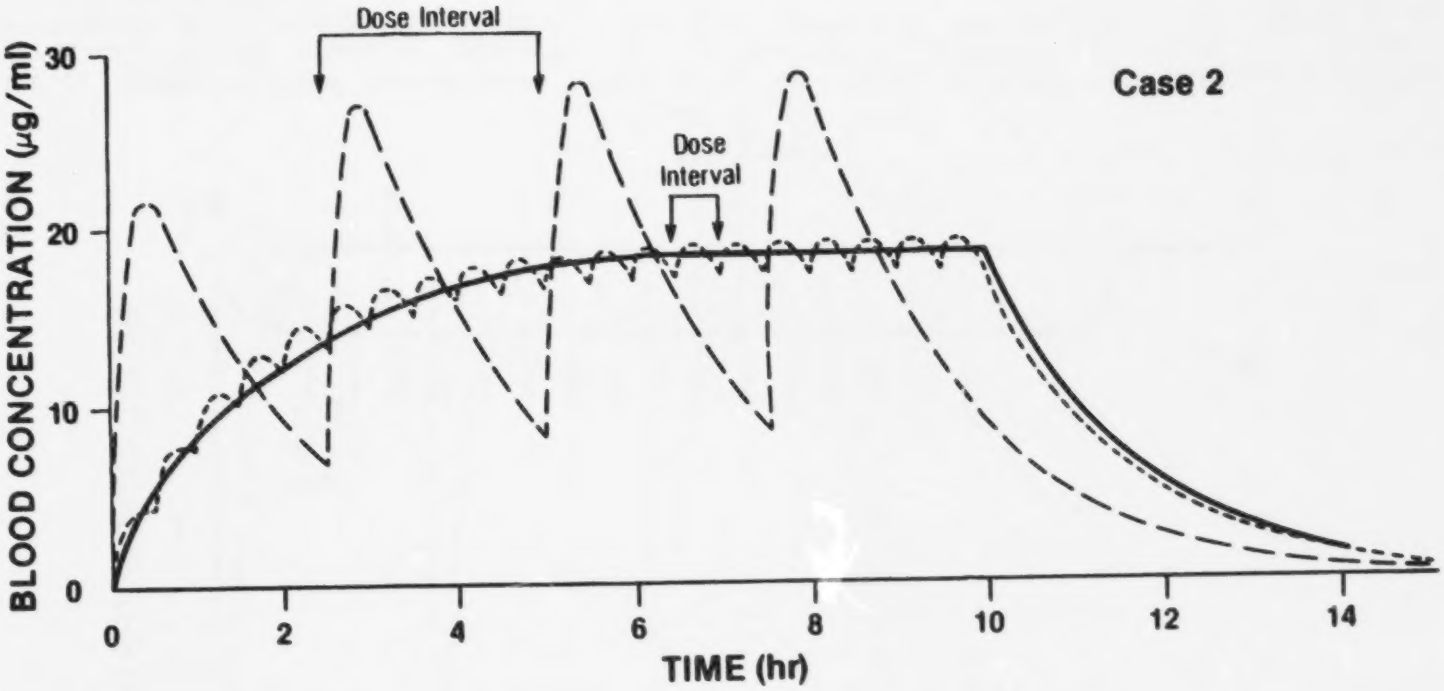


FIGURE 15

Case 2. Temporal blood concentration relationships for uptake by inhalation and gastrointestinal routes. (——) 10-hour inhalation exposure. (— —) four equal divided doses, orally over 10 hours. (----) 20 equal doses, orally over 10 hours. The rate coefficients for absorption and elimination for Case 2 are given in Table 5.

Source: Withey, 1983

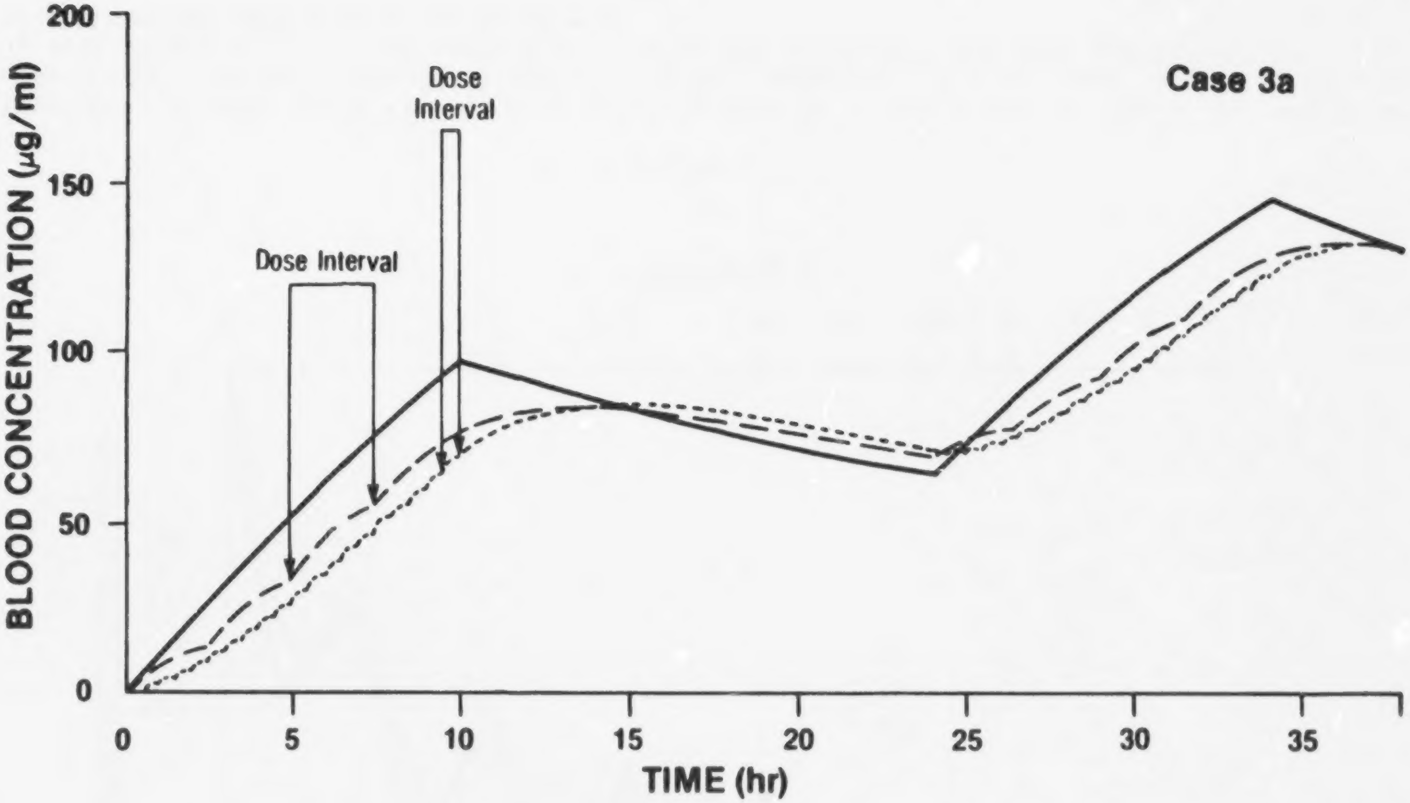


FIGURE 16

Case 3a. Temporal blood concentration relationships over 37 hours for uptake by inhalation and gastrointestinal routes. (——) 10-hour inhalation exposure. (— —) four equal divided doses, orally over 10 hours. (----) 20 equal doses, orally over 10 hours. The rate coefficients for absorption and elimination for Case 3 are given in Table 5.

Source: Withey, 1983

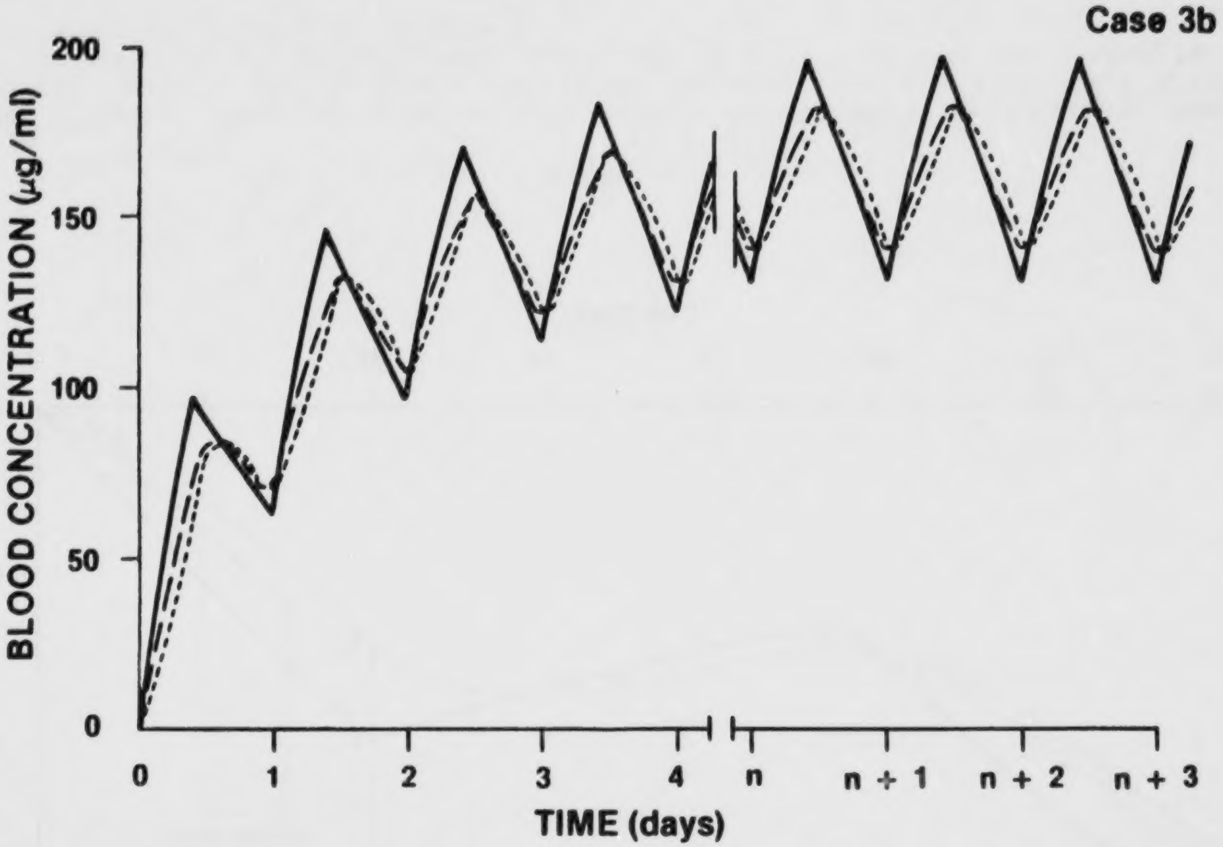


FIGURE 17

Case 3b. Temporal blood concentration relationships to steady state for uptake by inhalation and gastrointestinal routes. (——) 10-hour inhalation exposure. (— —) four equal divided doses, orally over 10 hours. (----) 20 equal doses, orally over 10 hours. The rate coefficients for absorption and elimination for Case 3 are given in Table 5.

Source: Withey, 1983

TABLE 5

Significant Parameters for the Dosing Routes and Regimens Illustrated in Figures 14-17

Parameter	Inhalation			Oral					
				4 doses			20 doses		
	Case 1	Case 2	Case 3	Case 1	Case 2	Case 3	Case 1	Case 2	Case 3
Absorption Rate Coefficient (hr^{-1})	11.2[a]	11.2[a]	11.2[a]	46.1	5.99	0.288	46.1	5.99	0.288
$t_{1/2}$ (min.)	--	--	--	0.9	6.9	144	0.9	6.9	144
Elimination Rate Coefficient (hr^{-1})	4.61	0.599	0.0288	4.61	0.599	0.0288	4.61	0.599	0.0288
$t_{1/2}$ (min.)	9.0	69	1440	9.0	69.0	1440	9.0	69	1440
Total AUC (0-24 hours)	24.3	186.9	3888	24.30	186.9	3888	24.30	187.0	3888
AUC (0-10 hours)[b]	23.77	55.8	1642	24.30	172.0	1665	24.30	157.4	1558
Limiting Maximum Concentration (µg/mℓ)	2.43	18.69	194.3	21.68	28.72	182.1	4.87	19.36	181.7
Limiting Minimum Concentration (µg/mℓ)	2.43	18.69	129.8	0.0003	8.96	139.1	0.69	17.49	143.1

[a] The units for the inhalation uptake rate are mg/ℓ/hr.

[b] The units for AUC are µg/hr/mℓ and the areas were determined for steady state conditions.

In Figures 16 and 17 (Case 3a and 3b), the elimination rate has further been reduced by a factor of 20 compared to that of Case 2. There is very little difference in the temporal relationship of blood levels after dosing, although steady state levels are not reached for 4 or 5 days of repeated dosing (see Figure 17). There is, in this example, evidence for bioaccumulation.

These illustrations, which could be extended to accomodate specific compounds with known pharmacokinetic parameters for any pharmacokinetic model, suggest that the larger the number of oral doses, the more closely the concentration time curve will correspond to an inhalation exposure. Route extrapolation will be more applicable for compounds with very slow elimination rates, like some pesticides and heavy metals, than for substances that are rapidly metabolized or excreted. It should be noted that, in every case where the different routes and regimens were compared, the area generated under the blood-level/time curve was the same. The area under the blood-level/time curve may not be a good indicator of equivalent systemic toxicity, especially when elimination is rapid. Finally, where the toxic insult is generated at the site of uptake rather than as a consequence of uptake to the systemic circulation, as in the case of pulmonary exposure to arsenic or manganese, route extrapolation using any kind of modeling approach is precluded.

CRITIQUES

DR. ELLEN O'FLAHERTY

The Stokinger-Woodward method, which has been used to convert exposure data from inhalation studies to comparable oral or intragastric exposures, has an additional limitation that was not specifically discussed by Dr. Withey, although he has alluded to the paucity of precisely measured absorption factors. It is well known that deposition, and subsequently absorption, are strongly dependent on particle size. Figure 18 is taken from the report of an extensive and carefully integrated series of studies of human absorption and deposition of lead, especially of lead from automobile exhaust (Chamberlain et al., 1978). It shows deposition in lung of wind tunnel aerosols generated by burning gasoline containing radiolabeled (^{203}Pb) tetraethyllead in an automobile engine. Particle size of the aerosols was varied by varying the rate of dilution and entry of the engine exhaust into the wind tunnel air flow. The diffusion mean equivalent diameter (DMED) is given for each of the three deposition curves. The percentage of the inhaled aerosol deposited in the lung was measured by two different methods, the results of which agreed well: by difference (inhaled ^{203}Pb minus exhaled ^{203}Pb), and by external gamma ray spectrometry of the ^{203}Pb deposited in the lungs. Figure 18 shows the strong dependence of deposition both on breathing cycle and on DMED. At the 4-second breathing cycle of standard man, deposition varied from 24-68% within this size range of very small particles.

Interestingly, further work by Chamberlain et al. (1978) showed that once deposited, lead was absorbed into the systemic circulation at remarkably similar rates irrespective of particle size (in the submicron range),

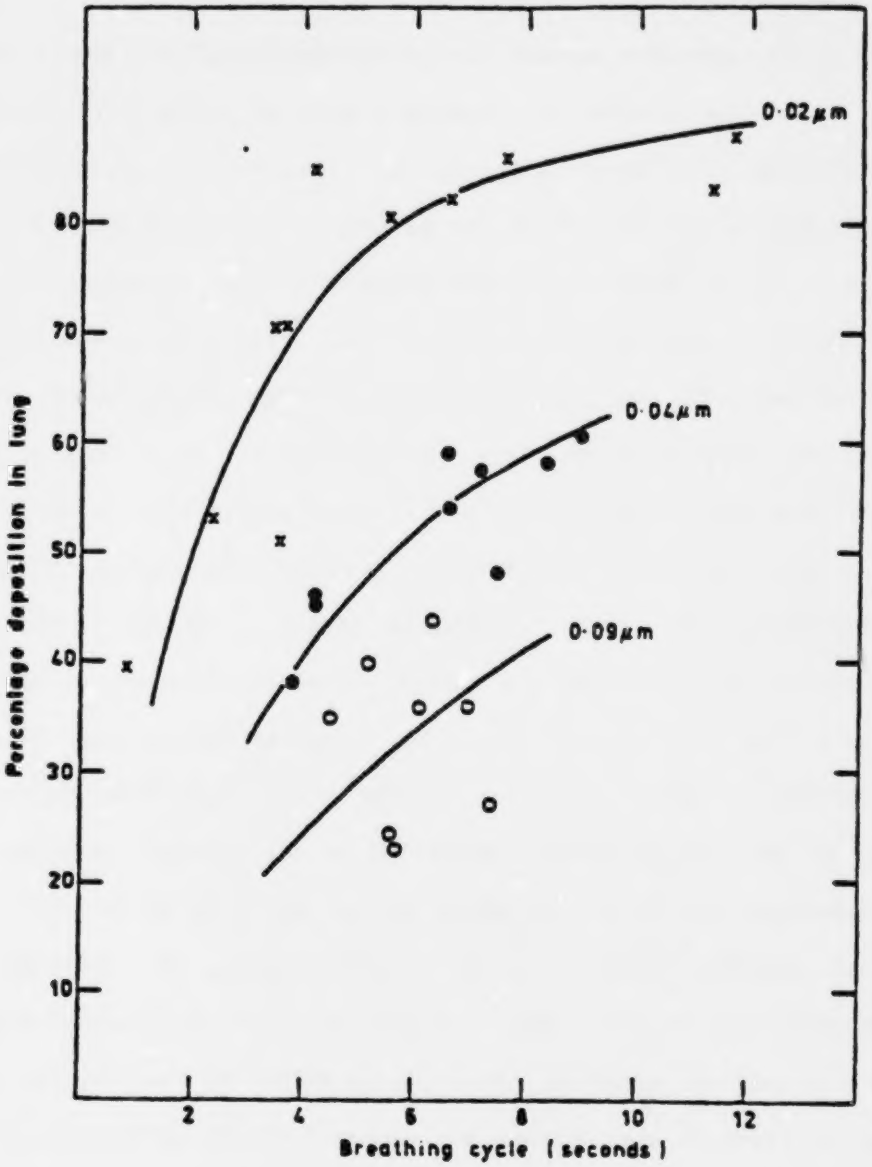


FIGURE 18

Deposition in Lung of Wind Tunnel Aerosols

Source: Chamberlain et al., 1978 (Copyright permission granted)

chemical nature, or concentration in the lungs. These observations are illustrated in Figures 19-21. Eight subjects participated in these experiments.

Figure 19 is a comparison of the lung clearances of ^{203}Pb-labeled aerosols of different chemical composition and DMED. Clearances are very similar up to 10 hours, with some differences (related to aerosol type) appearing at later times. By 30 hours, less than 10% of all aerosols but the carbonaceous exhaust remained in the lung.

Figure 20 shows the appearance of ^{203}Pb in the blood during clearance of several of the aerosols (see Figure 19) from the lung. These data are compared with the results of an earlier study (the curve labeled "1973/74 exhaust aerosols"), and with the percentage of a single dose of $PbCl_2$ in saline remaining in the blood at different times after intravenous injection. The similarity of the peak percentages, achieved at about 30 hours after either intravenous injection or cessation of inhalation exposure, was used by the authors to support their conclusion that much of the ~30% of ^{203}Pb originally deposited in the lungs but subsequently unaccounted for, had in fact been absorbed into the systemic circulation before distribution into peripheral tissues.

Finally, Figure 21 illustrates the lack of dependence of lung clearance on the amount of lead deposited in the lungs. For comparison, the authors noted that continuous human exposure to 1 μg Pb/m^3 with 50% retention would lead to daily deposition in the lungs of 8 μg of lead. In this study, the percentage of deposited lead found in the blood 48 hours later was only marginally affected (not significant at the 10% level) by the amount originally deposited.

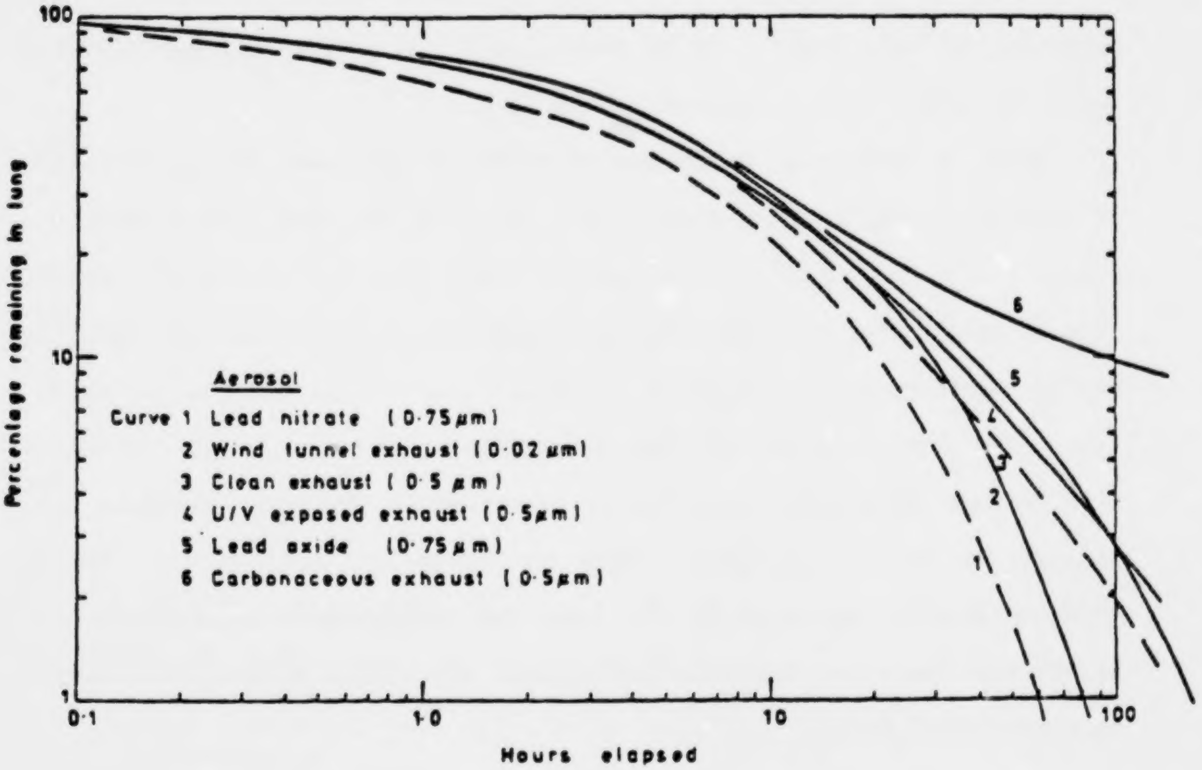


FIGURE 19

Comparison of the Lung Clearance of Various ^{203}Pb-Labeled Aerosols

Source: Chamberlain et al., 1978 (Copyright permission granted)

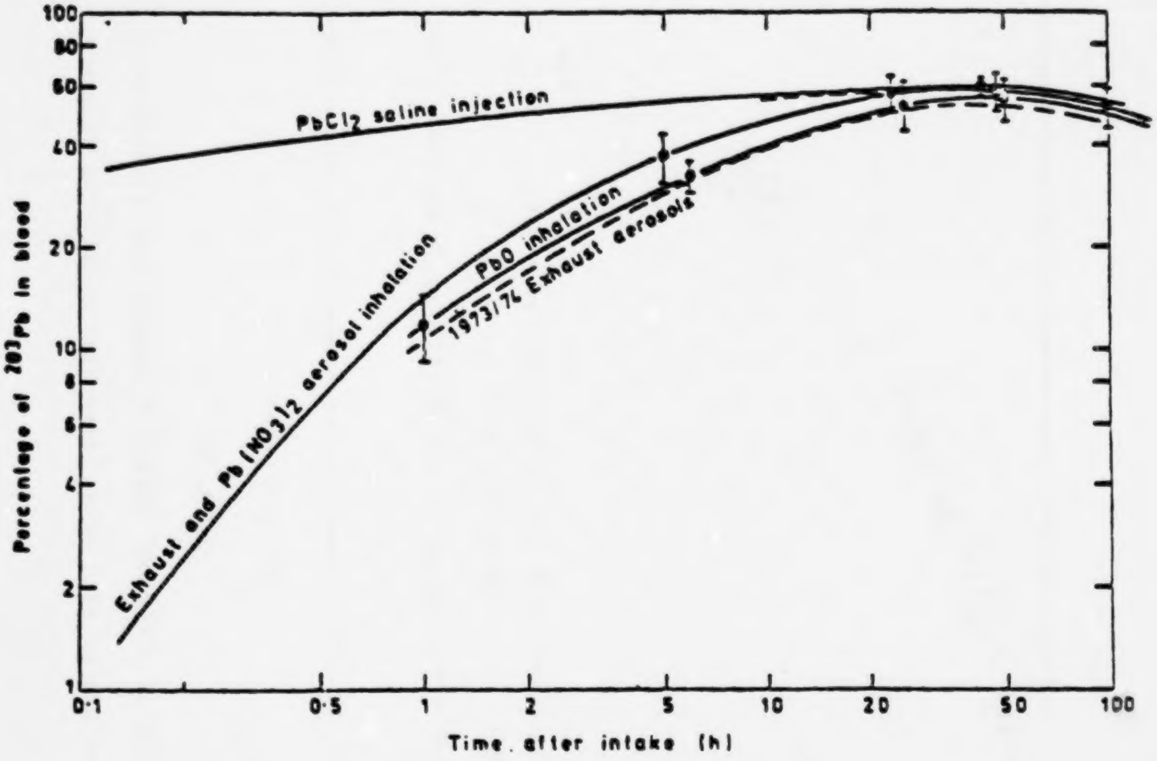


FIGURE 20

Levels of ^{203}Pb in Blood Following Inhalation of Exhaust Oxide or Nitrate Aerosols, or Injection of $PbCl_2$.

Source: Chamberlain et al., 1978 (Copyright permission granted)

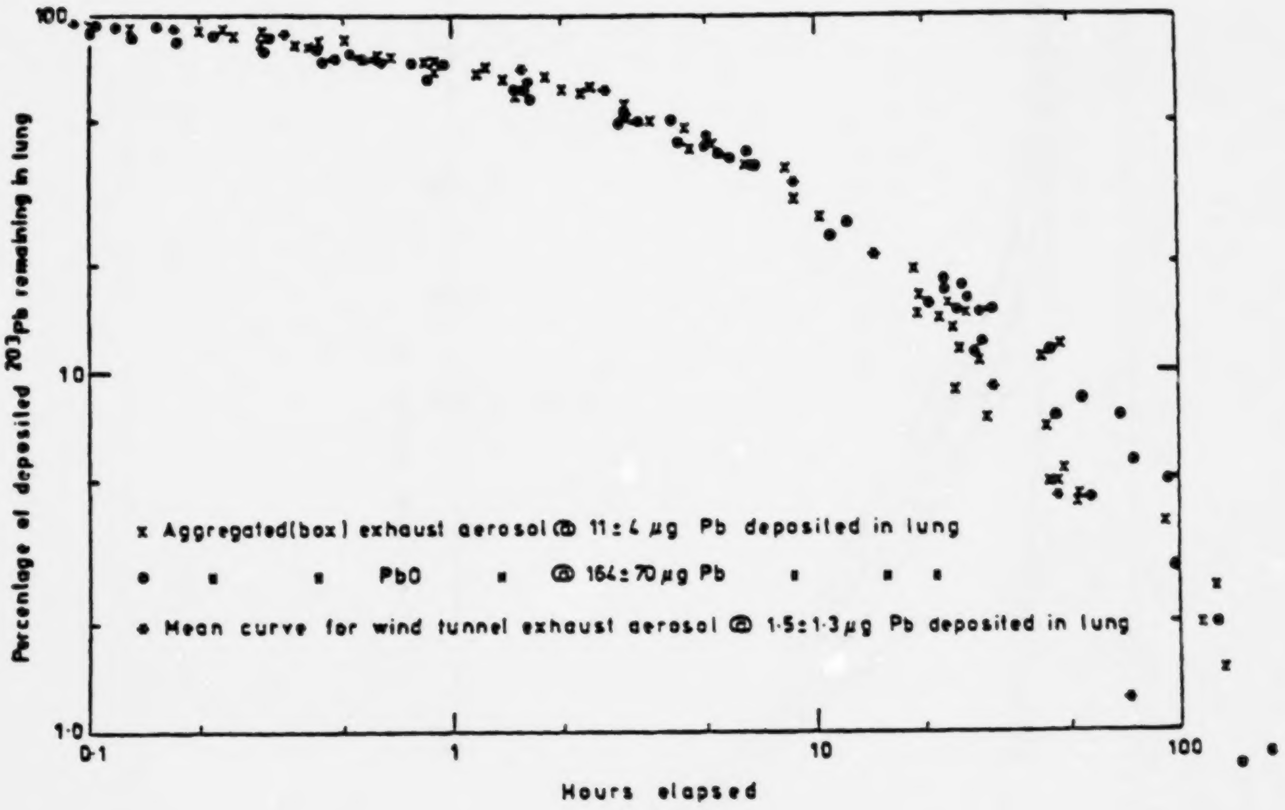


FIGURE 21

Lung Clearance at Different Levels of Stable Lead Deposition

Source: Chamberlain et al., 1978 (Copyright permission granted)

DR. JULIAN ANDELMAN

The principal question addressed by Dr. Withey in his presentation was the extent to which human or animal data on the effects or uptakes via one route of exposure can be extrapolated to another route. One example mentioned was the Stokinger-Woodward approach in which ACGIH TLVs for air exposures have been used to derive similar limitations for oral route uptake via drinking water. Dr. Withey discussed the impact of dose frequency and absorption and pharmacokinetic phenomena on body burdens. He discussed a paper by Perucca et al. (1978) to evaluate the equivalency of oral versus intravenous dosing.

The short-term (repeated dose) equivalencies and fluctuations are of interest and importance, although perhaps more from the point of view of simply demonstrating that routes can be compared. There are instances where an exposure of hours or less could be of importance, such as a tank car spill, but a more important need is probably to consider substantially longer exposures, as in Withey's case 3 example, at least when attempting to assess the impacts at hazardous waste sites. Fluctuations around a constant exposure should, however, not be set aside entirely, as they could, for example, be important for children playing periodically in the vicinity of a chemical dump site. Nevertheless, the discussion below will focus primarily on comparisons of routes in the steady state; that is, when the exposure is sufficiently long so that the uptake, metabolism, excretion and other kinetic phenomena generally occur sufficiently fast compared to the exposure period. The steady state is simply defined here as the achievement of a relatively constant body burden (e.g., blood concentration) and an equal rate of uptake and elimination.

The important absorption, excretion and pharmacokinetic issues relevant to extrapolation from one route of exposure to another, particularly inhalation to oral or vice versa, can be stated as follows:

1. Is a single or two-compartment model with zero- or first-order absorption kinetics and first-order metabolism and excretion valid? What are the implications of such a model?
2. Can LD_{50}/LC_{50} ratios be used to estimate other relative effects for air and water at much lower exposure concentrations; that is, do the kinetics change at lower exposures?
3. Can homologous series or other chemical comparisons be used to estimate blood levels (and, hence, effects) by different routes of exposure?
4. Are "first pass" effects important in long-term, steady-state systems?
5. If a metabolite is the active toxic agent, will a proportionality of dose and blood concentration be obtained?

All these issues relate to the proportionality between doses via different routes and the predictability of effects by comparison of routes of uptake. In order to examine these questions it is useful to consider the relevant aspects of the model, with particular emphasis on the single compartment model. This will be considered for two types of uptake.

Zero-Order Uptake

Here, zero-order uptake will be defined as uptake that is rapid, complete, and independent of concentration, i.e., the total amount of chemical inhaled or ingested is absorbed across the alveolar membrane or GI mucosa. In this case, nevertheless, the daily absorbed amount is still proportional to the pollutant concentration in the air or water, simply because the amount inhaled or ingested is a product of concentration and volume intake, typically about 20 m^3 air/day for a 70 kg adult male, or 2 ℓ of drinking water per day. These analytical relationships for the single-compartment model with such a zero-order uptake are shown in Table 6. The model assumes that metabolism and excretion are first order with respect to the blood concentration. As shown in Table 6, in the steady state for either air or GI tract absorption, the blood concentration is proportional to the concentration of the chemical in the air. At the same time, if one wishes to compare the steady state blood concentration due to lung absorption versus GI tract absorption, these are proportional to the relative concentrations in air and water. The implication of this model is that for complete absorption by the two routes, the biological effects associated with blood concentrations are proportional to the intake quantities.

First-Order Uptake

The steady state, single-compartment model considered in Table 7 assumes that the uptake across the alveolar membrane or GI mucosa corresponds to first-order kinetics; that is, the rate of uptake is proportional to the concentration in the air or water. It differs, however, from the zero-order situation in that now the proportionality constant is a true rate constant, unknown a priori, and not simply equal to the daily volume of air or water ingested. In addition to adsorption, the model has also incorporated a first-order desorption from the alveolar membrane.

For the steady state via the lung route, the blood concentration is proportional to the air concentration. One important point that derives from the model is that the proportionality constant is not simply equal to that predicted by Henry's Law. Rather, it predicts a lower steady state concentration in the blood than would be expected at a true equilibrium. This is a consequence of the fact that there are other pathways (metabolism and excretion), in addition to simply desorption back into the lungs via the alveolar membrane.

TABLE 6

Single Compartment Model - Zero-Order Uptake

---

1. VIA LUNG

The daily uptake is proportional to the air concentration:

$$I_a = C_a \times V_{da}$$

The rate equation for the daily change in blood concentration is:

$$dC_b/dt = I_a/V_b - k_m \times C_b - k_e \times C_b$$

In the steady-state dC_b/dt is zero. Therefore,

$$C_b = (V_{da}/V_b) \times C_a/(k_m + k_e)$$

2. GI TRACT

In the steady-state one obtains a similar relationship:

$$C_b = (V_{dw}/V_b) \times C_w/(k_m + k_e)$$

3. COMPARING LUNG AND GI ABSORPTION

To obtain the blood concentration ratios via air and water routes, one simply divides the final equation in 1 and 2:

$$C_{b\text{-air}}/C_{b\text{-water}} = (V_{da} \times C_a)/(V_{dw} \times C_w)$$

Definitions:

C_b = the blood concentration of an absorbed chemical
C_a = air concentration
C_w = water concentration
V_{da} = daily volume of air intake
V_{dw} = daily volume of water intake
V_b = blood volume
I = daily mass intake of a chemical via air route (I_a) or water (I_w)

k = first order rate constant for absorption (k_a), excretion (k_e), or metabolism (k_m). In the case of absorption one can have either absorption by the lung (k_{a-L}) or the GI tract (k_{a-GI}).

k_d = first order desorption constant, usually from the lung and relevant to the model for first order uptake via the lung.

TABLE 7

Single Compartment Model - First-Order Uptake*

1. VIA LUNG

Following the approach shown in Table 6:

$$dC_b/dt = k_{a-L} \times C_a - k_d \times C_b - k_m \times C_b - k_e \times C_b$$

In the steady-state $dC_b/dt = 0$. Therefore,

$$C_b = k_{a-L} \times C_a/(k_d + k_m + k_e)$$

2. GI TRACT

By direct analogy, one can also show:

$$C_b = k_{a-GI} \times C_w/(k_d + k_m + k_e)$$

3. COMPARING LUNG AND GI ABSORPTION

To obtain the blood concentration ratios via air and water routes, one simply divides the final equation in 1 and 2:

$$C_{b-air}/C_{b-water} = (k_{a-L} \times k_{a-GI}) \times (C_a/C_w)$$

---

*See Table 6 for definitions of terms

As in the zero-order model, the GI steady state system has the same form as that for air uptake. This then leads to a comparison of blood concentrations for these two routes, as shown in Table 7, which indicates that their relative blood concentrations are proportional to the ratio of concentrations in the air and water. This is the same result as in the zero-order case. However, the proportionality constant is a true ratio of first-order rate constants and cannot be predicted a priori.

Implications and Conclusions

Both the zero- and first-order absorption kinetics indicate a proportionality between blood concentrations and dose in the steady state. This in turn implies a proportionality between air and water concentrations that should remain constant among effects. However, if the absorption kinetics are first rather than zero order, this proportionality is not predictable a priori. As discussed by Dr. Pepelko in the following section on multiple route exposure, an article by Pozzani et al. (1959) has compared inhalation-route LC_{50} values to those of oral LD_{50} for a strain of rat. These values were not derived from long-term studies, so there is some doubt whether steady state was obtained in each case. Nevertheless, about half these chemicals had relatively equal values of LC_{50}/LD_{50} and four -- all chlorinated chemicals -- had substantially lower values. Such data indicate that there would be some value in compiling and analyzing similar data, so as to be able to test further the proportionality of doses by these two routes, as well as to establish whether there is any systematic impact of chemical type. It would be of particular interest to see if the proportionality of dose would hold for different effects, particularly those that might be manifested at substantially different dose levels.

Another set of related concerns involves the question of "first-pass" effects and what impact there is on the kinetic relationships if a metabolite is the active toxicant. If the chemical of primary exposure is also the toxicant, it need not follow in chronic exposure that a first-pass metabolic effect eliminates the concentration in the blood completely. That is, there may be recirculation at a constant concentration, and the kinetic treatment in Table 6 deals with such metabolism. Similarly, an active metabolite might also circulate at constant concentration. Thus, in both these instances, the blood and, hence, the toxic effect could be proportional to the concentration of the chemical in the water or air to which there is exposure.

Conclusions

1. Both zero- and first-order absorption imply proportionality between intake concentration and blood levels.
2. For zero-order absorption, dose estimation is "simple."
3. For first-order absorption, relative route dose estimation requires either experimental kinetic data or can be deduced from known toxicological data (e.g., LC_{50} vs. LD_{50}).
4. Henry's Law estimate of blood concentrations in equilibrium with air concentrations may be invalid in steady state (too high a value of C_B).
5. If metabolism is first-order kinetics, conclusion (1) holds. Thus, "first-pass" effects need only address the location of the target site, not the question of proportionality. In long-term steady state, this issue may be unimportant.

DISCUSSION

DR. MYRON MEHLMAN

This approach is essential for modeling. However, in the absence of data it will remain very limited for a long time to come.

DR. MAGNUS PISCATOR

Concerning Dr. Withey's discussion of the limitations of the Stokinger-Woodward method at the beginning of his presentation, it should be added that TLVs are not supposed to protect every worker. There is generally no safety factor in a TLV. Susceptible individuals are not protected.

DR. SHELDON MURPHY

Although I view the issue of this conference as one of assessing the potential for toxic interactions resulting from exposure to a mixture of chemicals, I do not believe we will ever determine ADIs of mixtures. Instead we will need to know how and how much one or more chemicals will affect the toxicity of the most biologically reactive or predominant-quantity chemicals in the waste dumps and/or the surrounding environment. Therefore, if we know the mechanism of action, the routes and mechanisms of metabolism, and the rates and routes of absorption and excretion of individual chemicals, we may be able to use rate constants, affinity constants, and potency of intrinsic activity at sites of injury for individual chemicals to predict the likelihood of more (or less) hazard in the presence of other chemicals. We should strive to obtain or encourage acquisition of appropriate basic data. In the meantime, EPA will have to work with the data available. For many of the common substances in waste dumps, there may in fact be more data in the published literature on these basic characteristics than on the actual toxicity of interactions, and therefore some theoretical predictions of added hazard from combinations may currently be possible.

As has been encouraged at this workshop, EPA should strive to use all available information to attempt an integrated assessment of hazard.

DR. RICHARD KOCIBA

The inherent limitations of the Stokinger-Woodward Basis for Extrapolation were clearly identified in the discussion and should be accommodated. Any regulatory plan should have the flexible basis to utilize pharmacokinetic data when they are available, because the most valid estimate of interspecies extrapolation is based on the concentrations at the critical target tissue level, rather than concentration in ambient air or other bases.

DR. HARRY SKALSKY

The Stokinger-Woodward model has served as a necessary approximation of route-to-route extrapolation, but as Dr. Withey suggests, it is time to become more sophisticated. Indeed, a goal should be set to establish models that can extrapolate on the basis of concentration of toxicant at the critical target tissue.

GENERAL COMMENTS

- Pharmacokinetic models may fit the data for one species but the variations can be very large among different species.
- The number of apparent compartments required for adequate model fitting varies depending upon the dose at which the compound is administered. For low doses, one-compartment models may be appropriate. At the highest tolerable dose, a three-compartment model may be appropriate.
- The continued use of the Stokinger-Woodward model to derive limits on uptake from water from TLVs for air exposure should be questioned. TLVs may be too high when applied to the general population, since TLVs are defined as that level which will not cause some degree of harm to the working population.
- It should be noted that Dr. Stokinger did not expect his paper to be taken so seriously; the model was only suggested for an emergency situation.

REFERENCES

Barr, W.H. 1968. Principles of biopharmaceutics. Am. J. Pharm. Educ. 32: 958-981.

Chamberlain, A.C., M.J. Heard, P. Little, D. Newton, A.C. Wells and R.D. Wiffen. 1978. Investigations into Lead from Motor Vehicles. Report No. AERE-9198. Environmental and Medical Sciences Division, AERE, Harwell, England.

Federal Register. 1979. U.S. Environmental Protection Agency, Water Quality Criteria, Requests for comments, Part V. 44(52): 15926-15981.

Perucca, E., G. Gatti, C.M. Frigo and A. Crema. 1978. Pharmacokinetics of valproic acid after oral and intravenous administration. Br. J. Pharmacol. 5: 313-318.

Pozzani, U.C., C.S. Weil and C.P. Carpenter. 1959. The toxicological basis of threshold limit values: 5. The experimental inhalation of vapor mixtures of rats, with notes upon the relationship between single dose inhalation and single dose oral data. Ind. Hyg. J. 20: 364-369.

Stokinger, H.E. and R.L. Woodward. 1958. Toxicologic methods for establishing drinking water standards. J. Am. Water Works Assoc. 50: 515-529.

Withey, J.R. 1976. Pharmacodynamics and uptake of vinyl chloride monomer administered by various routes to rats. J. Toxicol. Environ. Health. 1: 381-394.

Withey, J.R. 1983. Approaches to route extrapolation, Chapter 15. In: Principles for the Evaluation of Toxic Hazards to Human Health, R.G. Tardiff and J.V. Rodricks, Ed. Plenum Publications Inc., New York, NY. (In press)

SYSTEMIC TOXICANTS

Multiple Route Exposures

Presentation:	Dr. William Pepelko ECAO, OHEA, U.S. EPA
Critique:	Dr. Myron Mehlman Mobil Oil Corporation
Critique:	Dr. James Melius National Institute for Occupational Safety and Health

PRESENTATION

DR. WILLIAM PEPELKO: MULTIROUTE EXPOSURE

Present Approach

Multiroute exposure is considered in the "Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses" (45 FR 79354). For noncarcinogens, ADIs and criteria are calculated from total exposure data that include contributions from the diet and air. The criterion [C] for noncarcinogens is calculated using the following equation:

$$C = \frac{ADI - (DT + IN)}{2\ \ell + (0.0065\ \mathrm{kg} \times R)}$$

where 2 ℓ is assumed daily water consumption, 0.0065 kg is assumed daily fish consumption, R is bioconcentration in units of ℓ/kg, DT is estimated non-fish dietary intake, and IN is estimated daily intake by inhalation.

If experimental data are not available to estimate IN, then assumptions concerning ambient air concentrations, absorption percentage, etc. are made. The volume of air respired can be estimated using standard equations. In the case of the rat for example, the number of cubic meters breathed per day is $0.105\ [W/0.113]^{2/3}$, where W = body weight in kg.

Possible Approach

Any method used, in order to be valid, must estimate the concentration of the test substance at the critical target organ. Assuming that the site of exposure is not the primary target organ, then the relative contribution from each source may be summed. A summation of intake from each route, however, may lead to serious errors since the effectiveness of a chemical can vary considerably depending upon route of exposure, even with absorption of equivalent amounts. For example, some chemicals such as lidocaine are removed from the circulation on the first passage through the liver, thus rendering them ineffective via the oral route.

When adverse effects are not essentially at the site of entry and residence time in the body is several hours or more, then route equivalence can be assumed to be based on expected blood concentrations. The net dose (d_n), for a given species is then the sum of the absorbed doses for the various routes.

$$d_n = d_1 r_1 + d_2 r_2 + \cdots d_i \, r_i$$

where r is the absorption fraction and 1,2 ··· i refer to various routes of exposure. Human exposures are generally limited to oral, inhalation and occasionally dermal exposures. Experimental animals, however, may be exposed via intraperitoneal, intravenous, subcutaneous and other routes. An assessment based on an animal ADI, for example, then assumes that acceptable exposure levels are those in which ADI is greater than d_n.

In the case of chemicals that are rapidly removed by the liver or rapidly metabolized by peripheral tissues (cyanide for example), the concentration at the critical target organ may vary depending on route of entry. Then the dose for each route must be related to acceptable intake for that route, and the resulting scaled dose values summed to determine acceptable exposure. For the oral route the dose value would equal daily dose d_1 divided by the oral ADI. For the inhalation it would equal d_2/8-hour TLV where d_2 equals concentration in mg/m^3 x hours exposed/day divided by 8. If necessary a dermal exposure value could be calculated similarly. Acceptable exposure levels would be those in which $d_1/ADI + d_2/TLV$ was less than one.

Finally, in the case where the target organ differs depending upon route of exposure the doses are not summed and the treatment is the same as that for two different chemicals.

Issues

Some chemicals such as lidocaine are completely removed by the liver in a single passage. Thus, even with 100% absorption from the GI tract, none will reach the peripheral circulation. With inhalation or dermal exposure, peripheral regions will be exposed. Thus the extent of liver removal or alteration must be considered.

A test chemical with a short half-life may reach a steady state with continuous inhalation. Since intake via the oral route is likely to occur at irregular intervals, a steady state is less likely to be achieved. A single oral dose may also result in a greater peak exposure at the target organ than the same total dose via inhalation. In the case of many noncumulative poisons such as cyanide, the peak concentration is more important than the total dose.

In some cases the site of exposure is a primary target organ. This is often true with oral or dermal exposure. Should a separate ADI be estimated for each route? If so, should we sum $d_1/ADI_1 + d_2/ADI_2$, and is it a serious problem if the sum is greater than 1?

A large portion of inhaled particulate matter is deposited in the conducting airways, carried upward via the mucociliary ladder and swallowed. Thus a significant portion of inhaled particules or chemicals adsorbed on particles may enter the body via the digestive tract. Methodology must be modified to account for this.

Relative Effectiveness of Oral vs. Inhalation Exposures

The data from a study by Pozzani et al. (1959) were recalculated in order to show the variation in the effectiveness of oral vs. inhalation exposure for a series of organic chemicals. Since body weights were not given, it was assumed that the rats weighed 0.33 kg. Respiratory minute volume was estimated using the equation $I = 0.105\ (wt/0.113^{2/3}/m^3/day)$.

A 0.33 kg rat would thus inhale 72 ℓ in 8 hours. The absorption percentage was also not given and therefore not considered. It is likely, however, if absorption rate is high by one route it would also be high by the other. The data are presented in Table 8.

As can be seen, the mean ratio of LD_{50}/LC_{50} was 1.8. The largest ratio, however, was about 21 times greater than the smallest ratio. This was most likely due to several factors. A mean ratio of 1.8 suggested that most chemicals were absorbed more effectively from the lung. This is not unexpected since the human lung has a surface area of about 70 m^3. Liver detoxification may have decreased the relative effectiveness of some chemicals via the oral route. Finally, for chemicals with a very short half-life, a steady state may be reached via inhalation but not via the oral route. However, a higher peak level may be achieved via the oral route.

Thus while the relative effectiveness of the two routes of exposure varied by a factor of two or less for about two-thirds of the chemicals, a better knowledge of pharmacokinetics is required before doses from the two routes can be accurately combined.

TABLE 8

A Comparison of Lethal Oral Verus Inhalation Doses for a Series of Organic Chemicals[a]

	8-hour LC_{50} (g/rat)	Oral LD_{50} (g/rat)	Ratio LD_{50}/LC_{50}
Acetone	3.61	4.20	1.16
n-Butylacetate	2.93	4.97	1.69
Butyl alcohol	2.12	2.05	0.97
Butyl cellosolve	0.20	0.94	4.70
Cellosolve	0.53	2.05	3.86
Cellosolve acetate	0.87	2.57	2.95
Ethylene dichloride	0.29	0.21	0.72
Isobutanol	1.33	2.05	1.54
Isopropanol	1.99	3.57	1.79
Isopropyl acetate	3.64	5.73	1.57
Methanol	4.27	5.93	1.39
Methyl ethyl ketone	1.69	2.29	1.39
Methyl isobutyl ketone	0.84	1.87	2.23
Perchloroethylene	2.46	0.54	0.22
Propylene dichloride	1.01	0.56	0.58
1,1,2-Trichloroethane	0.39	0.19	0.49
			1.81[b]

[a] Source: Pozzani et al., 1959 (Copyright permission granted)

[b] Average ratio

CRITIQUES

DR. MYRON MEHLMAN

The assumptions involved in setting criteria levels are extremely soft. In most cases, the levels are significantly below natural background levels for known, naturally occurring substances such as benzene. These assumptions need to be reexamined.

The availability of sufficient data to estimate multichemical exposure by various routes is questionable. Some of the parameters which must be considered are:

- Metabolic fate
- Proliferative activity of target tissue at time of exposure
- Interaction with DNA or protein
- DNA repair
- Dose-time effect
- Age-associated decline in enzyme and hormone activity
- Age-associated decline of proliferative activity of epithelial cells in intestines and kidneys
- Age-associated disturbances in ability to repair

For exposure to single chemicals, exposure routes may be used interchangeably. For exposure to mixtures this is not true. Examples of the effects produced by exposure to industrial chemicals by different routes of exposure are shown in Tables 9 and 10. A comparison of the blood concentrations resulting from exposure to complex hydrocarbon mixtures by various routes is shown in Figures 22 and 23.

DR. JAMES MELIUS

It is important to differentiate between occupationally- and environmentally-derived criteria (i.e., TLV vs. ADI). For most chemicals the environmentally-derived criteria will be much lower than the occupational

TABLE 9

Percent Rats with Tumors Following Vinyl Chloride Exposure*

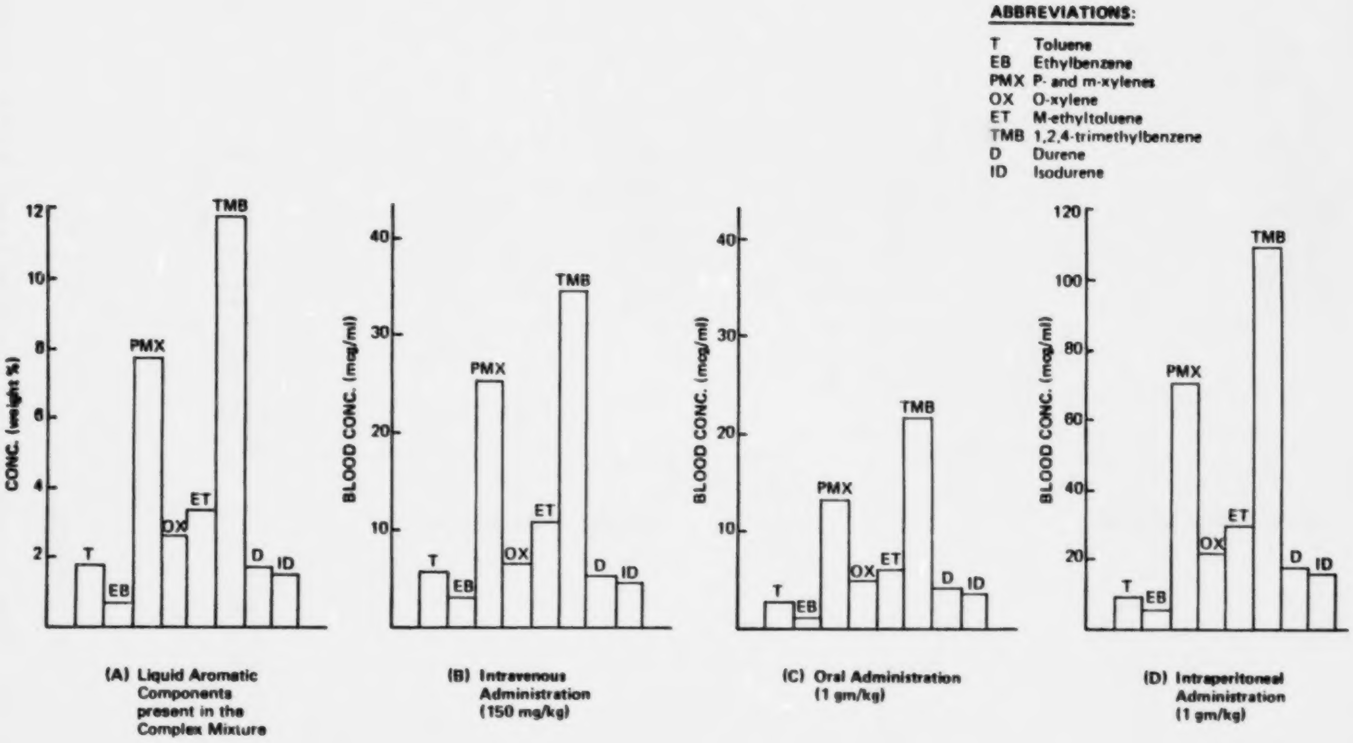
Daily Dose (mg/kg)	Duration	Percent Rats with Tumors in:						
		Zymbal Gland	Mammary	Liver	Kidney	Thymus	Abdomen	Other
ORAL								
50.0	120 weeks	1	6	20	2	1	1	37
16.6	120 weeks	2	7	11	4	0	0	25
3.3	120 weeks	0	5	0	0	0	1	10
0.0	120 weeks	1	5	0	0	0	0	10
INHALATION								
75.0 (25 ppm)	87 weeks	3	12	4	0	--	--	6
30.0 (10 ppm)	87 weeks	4	12	0	0	--	--	4
3.0 (25 ppm)	87 weeks	0	9	0	0	--	--	3
0.0	87 weeks	0	2	0	0	--	--	3

*Source: Maltoni, 1977 (Copyright permission granted)

TABLE 10

Percent Rodents with Tumors Following Benzene Exposure

Rodent	Daily Dose (mg/kg)	Duration	Percent Rodents with: Zymbal Gland Carcinomas	Percent Rodents with: Hemolymphoreticular Neoplasia	Reference
INHALATION					
Mouse	360 (300 ppm)	6 hours/day, 5 days/week for lifetime	--	20.0	Snyder et al., 1980
Mouse	0 (control)	--	--	5.0	Snyder et al., 1980
Rat	360 (300 ppm)	7 hours/day, 5 days/week for 85 weeks	5.8	--	Maltoni, 1982
Rat	0 (control)	--	0.3	--	Maltoni, 1982
ORAL					
Rat	250	daily, 4-5 days/week	12.3	7.7	Maltoni and Scarnato, 1979
Rat	50	52 weeks	6.9	3.4	Maltoni and Scarnato, 1979
Rat	0 (control)	--	0.0	1.7	Maltoni and Scarnato, 1979



(A) Liquid Aromatic Components present in the Complex Mixture

(B) Intravenous Administration (150 mg/kg)

(C) Oral Administration (1 gm/kg)

(D) Intraperitoneal Administration (1 gm/kg)

FIGURE 22

Comparison of Blood Concentrations Resulting from Intravenous, Oral and Intraperitoneal Exposure to a Complex Hydrocarbon Mixture.

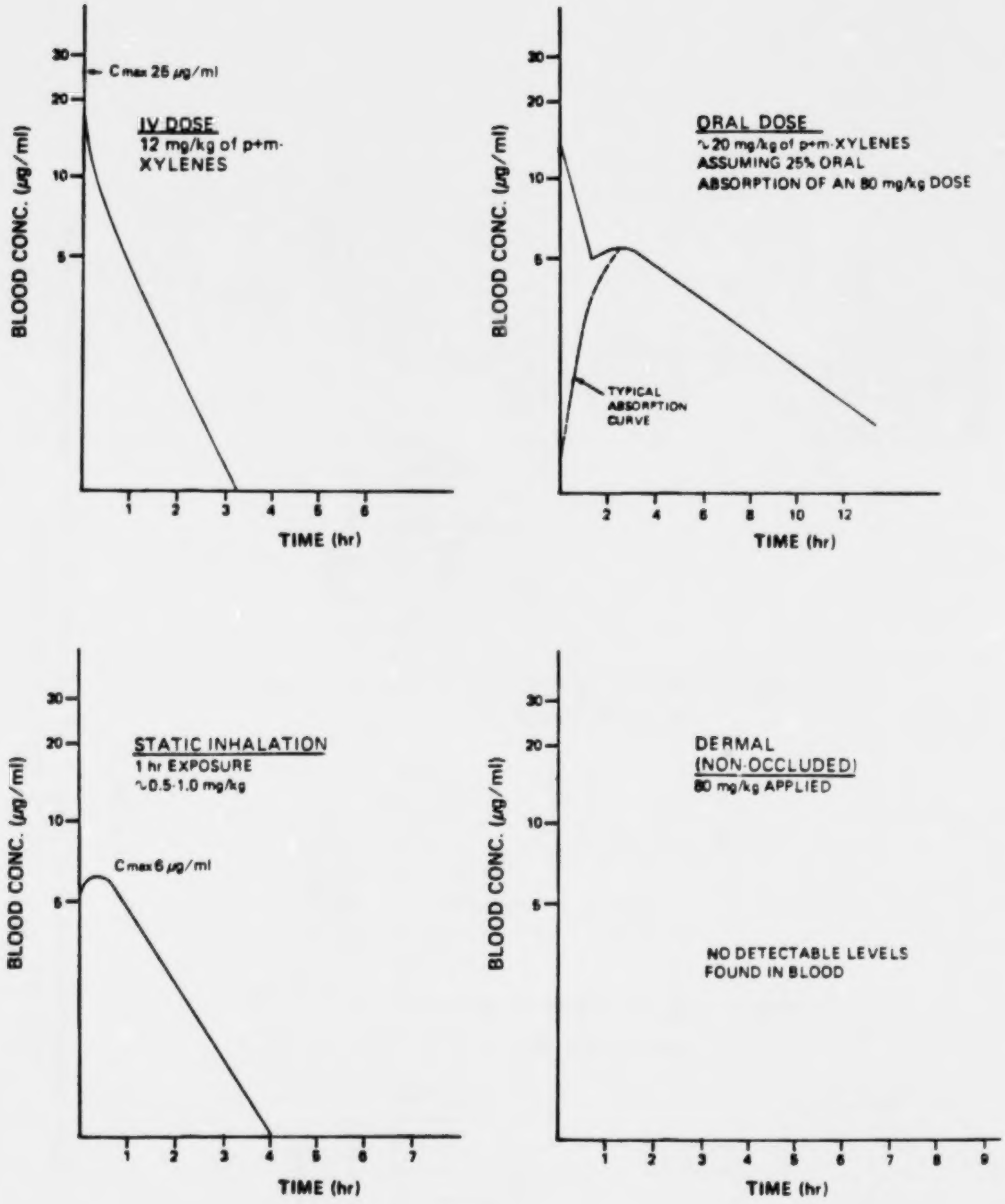


FIGURE 23

Blood concentration vs. time curves of p- and m-xylenes after various routes of administration of a hydrocarbon mixture (p- and m-xylenes constitute 8 wt. % of total hydrocarbon content).

criteria. For example, for chlorinated solvents, the occupational inhalation TLV allows a 1000-fold greater exposure than the environmental drinking water criteria. These differences reflect the historical and scientific bases for these criteria.

In dump sites and other hazardous waste situations, skin absorption is an important source of exposure. However, we have very little quantitative information on the amount of skin absorption and almost no criteria to determine if environmental surface levels are excessive. For example, NIOSH has investigated several situations involving electrical equipment failures where surface contamination with PCBs, dibenzofurans and dioxins were found. There are presently no criteria to evaluate the significance of these levels and to determine "safe" levels for cleanup purposes.

At a given site, different routes of exposure may vary in importance depending on the population of concern. For individuals involved in site investigation or response, air exposure will be a primary concern; for the surrounding community, water exposure may be more important. For each chemical at a waste site, a single route of exposure would be most important depending on the properties of the chemical and the characteristics of the waste site.

DISCUSSION

DR. RICHARD KOCIBA

Each dump site should be evaluated on a case-by-case basis to address the specific issues regarding multiple routes of exposure, interactions of component materials and the relative roles that inhalation, oral and dermal exposure play in the actual situations. One cannot generalize as to possible synergism, antagonism or additivity of multiple chemicals.

GENERAL COMMENTS

- It is unknown whether oral/inhalation dose-response curves are parallel.
- Vehicle effects may be important in exposure studies and must be considered.
- Nasal irritants need to be considered when discussing inhalation.
- If critical target organ is the point of entry, the intake from two routes of exposure would not be added.
- LC_{50} studies have been done on mixtures and dose-response curves have been developed.
- Two questions not addressed: Can routes of exposure be considered separately and then combined additively or in an equitoxic fashion? Does exposure by one route alter the toxic dose response by another route?

REFERENCES

Maltoni, C. 1977. Origins of human cancer. In: Proceedings of a Conference on Cell Proliferation. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. p. 119-146.

Maltoni, C. 1982. Myths and facts in the history of benzene carcinogenicity. Adv. Mod. Env. Toxicol. 4: 1.

Maltoni, C. and C. Scarnato. 1979. First experimental demonstration of the carcinogenic effect of benzene: Long-term bioassay on Sprague-Dawley rats by oral administration. Med. Law. 70: B-52.

Pozzani, U.C., C.S. Weil and C.P. Carpenter. 1959. The toxicological basis of threshold limit values: 5. The experimental inhalation of vapor mixtures of rats, with notes upon the relationship between single dose inhalation and single dose oral data. Ind. Hyg. J. 20: 364-369.

Snyder, C.A., et al. 1980. The inhalation toxicity of benzene: Incidence of hematopoetic neoplasm and hematotoxicity in AKR/J and C57Bl/6J mice. Toxicol. Appl. Pharmacol. 54: 323.

SYSTEMIC TOXICANTS

The Impact of Carcinogens in Risk Assessment of Chemical Mixtures

Presentation:	Dr. Robert McGaughy Cancer Assessment Group, U.S. EPA
Presentation:	Dr. Roy Albert New York University Medical Center Cancer Assessment Group, U.S. EPA
Presentation:	Dr. Debdas Mukerjee ECAO, OHEA, U.S. EPA

PRESENTATIONS

DR. ROBERT McGAUGHY: HOW DOES THE CAG ASSESS QUANTITATIVE RISKS?

Risk assessments generated by the EPA Carcinogenic Assessment Group (CAG) are intended to provide a rough estimate of the seriousness of an exposure situation. CAG estimates are not predictions and they do not provide an estimate of absolute risks. Rather, they serve to indicate which situations have negligible hazard and they provide a convenient framework for summarizing relevant facts and identifying research needs. They do not reflect the strength of the evidence.

In absence of information, CAG makes the following assumptions in assessing quantitative risks:

1. Lifetime incidence in humans is the same as in animals receiving an equivalent dose.
2. Dose in mg/surface area is equivalent between species.
3. Humans are as sensitive as the most sensitive animal species.
4. A linear, no-threshold model is the upper-limit response at low doses. No estimate is made if mechanism is known to be non-linear.
5. Lifetime incidence is proportional to the total lifetime dose received, averaged on a daily basis.
6. If an experiment is terminated early, lifetime incidence is estimated assuming that cumulative incidence increases as the third power of age.
7. The linearized multistage model is appropriate for extrapolation and the upper 95% confidence limit of the linear term is appropriate for expressing the upper-bound of potency.
8. The upper-bound risk is less plausible if there is no evidence of mutagenicity.
9. Human data are preferable to animal data as the basis for risk estimates.
10. Negative human data, if available, can be used to give an upper-limit of risk.
11. For human data, the method of analysis is tailored to the completeness and quality of data available. A model which is linear at low dose is used for extrapolation.

DR. ROY ALBERT: POLICY INITIATIVE

The purpose of this presentation is to describe a policy initiative launched by CAG and ECAO-CIN. The policy initiative deals with two matters: 1) the adoption of the International Agency for Research on Cancer (IARC) scheme for stratification of the weight of evidence for carcinogenicity, and 2) the use of mutagenicity data in the approach to quantitative risk assessment with special reference to water quality criteria.

The IARC Stratification Scheme

For the first 5 years after the adoption of the EPA guidelines for carcinogen risk assessment in May 1976, there was a strong impetus toward the regulation of any agent that showed even relatively modest evidence of carcinogenicity. The CAG has no formalized nomenclature for describing the weight of evidence and most frequently used the term "substantial" to characterize evidence that was more than marginal. However, within recent years, there has been increasing resistance to regulation, and, consequently, a greater need for a stratification of the weight of evidence for carcinogenicity. In examining the various options it was apparent that, although a number of schemes for describing the weight of evidence existed, only one had international acceptance and substantial usage: the approach developed and used by IARC for evaluation of close to 200 compounds. For this reason, it seemed appropriate for EPA to adopt the IARC scheme for stratifying the weight of evidence.

The proposal to adopt the IARC scheme was circulated within the EPA and to 22 of the country's outstanding experts in the field of oncology. All the outside experts who reviewed the IARC scheme were favorable to its adoption. There was also strong sentiment for retaining the original characterization of the "limited" category of evidence for situations where no decision could be made on whether the agent was a carcinogen. One reviewer commented that the distinction between the "sufficient" and "limited" categories should be sharpened and their regulatory significance clarified. Another commenter had reservations about limiting "sufficient" evidence to malignant neoplasms. Considering the response within and outside the agency to the proposed use of the IARC scheme and also the response of the participants at other ECAO workshops, it was apparent that adoption of the IARC stratification scheme would receive wide acceptance in the scientific community.

The Use of Mutagenicity Data and Quantitative Risk Assessment

The second of the two policy initiatives was to limit the use of the linear extrapolation model to agents that show evidence of being mutagenic. In terms of the water quality criteria, the proposal presented a range of water concentrations: the lower limit was based on the use of the linear extrapolation model at a risk level of 10^{-5}, and an upper concentration limit was based on a modified NOEL approach (a safety factor of 1000 applied to a 10% response). Guidance for each compound was given as to where in the concentration range the actual standard should be sought. The response of the outside experts was mixed. Some had very serious reservations about the approach, primarily on the grounds that not enough was known about the differences in the mechanism of action of mutagenic and nonmutagenic carcinogens. However, a substantial number of the experts supported the proposed approach.

The central argument in support of the proposed approach is that the mode of action of mutagenic compounds that is consistent with a linear non-threshold extrapolation model can be easily visualized as a quantal interaction of the carcinogen with DNA leading to a mutagenic event that is linked to a carcinogenic transformation. In the case of human bladder cancer, recent evidence supports this mode of action. By contrast, there is no mode of action for nongenotoxic carcinogens that has been postulated which would lead to single-hit non-threshold kinetics. The absence of even a speculative mode of action that would be consistent with such a pattern weakens the argument in support of the use of the linear non-threshold extrapolation model for nonmutagenic carcinogens. It was apparent from the meeting at Cincinnati that a number of the participants had the same sort of reservations as had been expressed by other experts in oncology. But a vote of the assembled group revealed that a majority supported the distinction between mutagenic and nonmutagenic carcinogens for the purpose of quantitative risk assessment.

DR. DEBDAS MUKERJEE: MECHANISMS OF CARCINOGENESIS IN RISK ASSESSMENT

Environmental factors, which include life style and environmental agents that man is exposed to, seem to be the major cause of most of the cancer in man. The mechanisms by which the normal cell is transformed by the environmental carcinogens to a malignant state are complex. Evidence from biochemical molecular analysis, _in vitro_ studies, animal bioassays and epidemiologic observations indicates that the problem of cancer lies in the regulatory dysfunction of the normal gene action. This dysfunction of the genetic material of cell results in the production of progeny of abnormally proliferating cancer cells. The neoplastic cells, in addition to acquiring a multitude of abnormal characteristics, lack certain essential basic properties of the normal cell. Since the reduction of exposure to environmental carcinogens shows great promise of reducing cancer incidence, it is essential to utilize a scientifically valid approach for regulating the carcinogens. Consequently, the major concern for the public health lies in whether the current knowledge of the mechanism of carcinogenesis can be utilized to determine cancer risk from exposure to environmental carcinogens.

One of the initial tasks for this mission is to detect the carcinogenic potentialities of the environmental agents for determining their cancer risk. Human epidemiologic observations have identified many of these environmental carcinogens. Animal bioassays have also aided in determining the carcinogenicity of chemicals. There are nearly two dozen human carcinogens which have been found also to be carcinogenic in animals. These assays have identified the carcinogenicity of many chemicals. In addition, chemicals once suspected of having carcinogenic potentialities because of structural similarities have been found to be noncarcinogenic in animal bioassays. To avert the massive expense, time and labor, other characteristics of carcinogenic agents are being studied for identification. Results from short-term test including microbial mutagenesis assays, sister-chromatid-exchanges, etc. have been utilized for selecting chemicals for long-term animal bioassays. Since carcinogens are supposed to affect the genetic materials and carcinogenesis is thought to be a process of mutation of the somatic cell, various short-term tests which can detect mutagenic characteristics of the chemical have been utilized. The carcinogens which express mutagenicity in short-term biological assays are thought to follow a genetic pathway in the carcinogenesis process (Ames et al., 1975). However, a small group of carcinogens consistently give negative response to mutagenesis assays. This has generated considerable confusion in the scientific community. It has even been suggested that the carcinogens which consistently fail to give positive response to indicate its mutagenic characteristics in short-term biological mutagenesis assays follow a nongenetic pathway to transform the normal cell to a neoplastic state (Weisburger and Williams, 1981).

Conclusive evidence that carcinogens affect the DNA may be derived from biochemical analysis of the DNA of the transformed cell (Lutz, 1979). Many carcinogens which respond negatively in short-term mutagenesis assays have been found to react with DNA. Such reactions can only be observed following sensitive biochemical analysis used in molecular biology.

That the basis of carcinogenesis lies in the alteration of DNA of the cell can also be supported by observations on carcinogen induced misrepair or incomplete repair resulting in deletion, addition or translocation of the base of the DNA molecule. The damage to the DNA strands by the carcinogen is very often repaired by enzymatic repair mechanisms to preserve the normal entity of the cell. When the enzymatic repair is also inhibited the cell acquires the neoplastic state (Setlow, 1978; Cleaver and Bootsma, 1975).

The active form of the carcinogens because of electrophilic nature binds covalently with DNA forming adducts (Miller, 1970). Carcinogens can form adducts with all the base residues in DNA. However, the N-7 position of the guanine, because of its location outside the helical axis (especially in Z-form DNA) (Rajalakshmi et al., 1982) becomes easily available for reaction with the active form of the carcinogen. Covalently bonded adducts between the electrophilic active form of the carcinogen and the electron rich DNA is an indicator of the mutagenic characteristic of the carcinogenesis. Ability of the carcinogen to form adduct with DNA is also an indicator of its carcinogenic activities. More than 80 carcinogens have been studied for their effectiveness of forming DNA adducts. Many of these carcinogens are barely detectable or even negative in Ames assay (Rajalakshmi et al., 1982). Furthermore, a measure of the DNA adduct formation with the carcinogen is also a measure of the quantity of the carcinogen induced in transforming the normal cell to the neoplastic state.

DNA adduct formation seems to be the most conclusive criterion for determining the actions of the carcinogen at the genetic level (Perera and Weinstein, 1982). Extreme cytotoxicity, extreme unstable nature of the reactive metabolite of the carcinogen, and absence of optimum enzymatic activation of the carcinogen to an active form are thought to be a few of the many reasons for the lack of expression of mutagenic activities in short-term biological assays. But for conclusive evidence it is essential to determine whether the chemical in question binds covalently with the genetic material and/or it mutates any gene of the cell. Covalent binding of the carcinogen with the DNA is a reflection not only of the degree of adduct formation, but also of the efficiency of specific DNA excision mechanisms.

Genetic and nongenetic mechanisms of carcinogenesis are not operating in a totally autonomous manner and simplistic separate theories can now be resolved by a unified concept that seems to fit all the evidence and define most of the aspects of carcinogenesis. A unified theory of carcinogenesis has recently been proposed (Ts'o, 1981). According to this concept the genetic material contains both DNA and the regulatory machinery which controls the expression and replication of DNA. Carcinogens affecting the regulators, usually thought of as nonmutagenic in biological short-term assays, can also affect the DNA and, therefore, may be characterized as mutagenic in nature. On the other hand, an effect on DNA can be expressed through the interaction with the regulator. Consequently, some genetic phenomenon can apparently look like a nonmutagenic event. Such dynamic interaction of the entire genetic material and the regulator, influenced by carcinogens irrespective of whether they are positive in biological mutagenesis assays or not, results in the heritable changes encountered in neoplastic cells.

The differentiation process for cancer development, first described as one of the primary nongenetic mechanisms of cancer development (Pitot and Heidelberger, 1963), now appears to be consistent with a genetic mechanism. Some hormones generally considered to be nonmutagenic have been found to react through a genetic mechanism. *In utero* exposure to diethylstilbestrol may result in vaginal cancer in the daughter 15 or more years later. This potent transplacental carcinogenic hormone and some of its metabolites have now been shown to bind covalently to DNA both *in vivo* (Lutz, 1979) and *in vitro* (Metzler, 1981). Furthermore, this hormonal carcinogen seems to induce sister-chromatid-exchanges in lymphocytes from pregnant and premenopausal women (Hill and Wolff, 1982). Asbestos fibers, though giving negative response in microbial mutagenic test systems, have also been suggested to interfere with DNA molecule. Synergistic effects relative to DNA binding for chrysotile and benzo(a)- pyrene have been observed in the human fibroblasts in culture when asbestos is added 24 hours prior to the hydrocarbon. Such DNA binding has been suggested to be due to interaction of the charged asbestos fibers with the DNA, thereby altering its electronic structure. This results in an increase in the number of sites available for binding of the hydrocarbon to the DNA (Hart et al., 1980). In addition, asbestos is also known to induce sister-chromatid-exchanges (Rom et al., 1983). From these observations it can be inferred that asbestos, a carcinogen negative in most of the mutagenesis assays, might also react through a genetic pathway. Carcinogenic metallic salts which are negative in mutagenesis assays can induce infidelity of DNA polymerase resulting in synthesis of aberrant DNA (Sirover and Loeb, 1976), DNA strand breaks (Robison and Costa, 1982) and formation of protein-metal-DNA complexes (Lee et al., 1982) in mammalian cells. Carbon tetrachloride and chloroform are potent animal hepatocarcinogens (IARC, 1979; NCI, 1976). Since these have consistently been found to respond negatively in microbial mutagenesis assays, it has been suggested, especially in the case of chloroform, that perhaps these carcinogens form tumors following strictly a nongenetic pathway. Phosgene, a highly reactive chemical, seems to be an important metabolite of carbon tetrachloride and chloroform. However, the metabolites of these hepatocarcinogens have been found to bind covalently with nuclear DNA (Diaz Gomez and Castro, 1980). Conversely, certain carcinogens like nitrosamines and 3-methylcholanthrene, which consistently give positive response in biological mutagenesis assays, also apparently seem to follow nongenetic pathways (DiMayorca et al., 1973; Prehn, 1964). These observations clearly indicate that covalent binding studies of the active form of the carcinogens conclusively demonstrate that carcinogens affect the genetic material to initiate a cell to the neoplastic state.

The multistage model for quantitative risk assessment from carcinogens assumes that the tumor develops from a single cell only after it undergoes a number of changes resulting in the dysfunction of the genetic material of the cell (Armitage and Doll, 1961). Utilization of this model for estimating risk from carcinogenic pollutants can be justified by the mechanism of carcinogenesis as proposed in the unified theory. One of the unique features of this model is that it gets its credence from epidemiologic data, animal studies and even *in vitro* studies of neoplastic transformation of cells. Most ideal risk assessment for carcinogens requires that human epidemiologic data and sufficiently valid exposure information are available for the compounds in question. In the absence of such observations, the data are analyzed by an alternate procedure to give an estimate of the linear dependence of cancer rates based upon the calculated lifetime average dose. If the epidemiology data show no carcinogenic effect when positive animal evidence is available, it is assumed that a risk exists but is smaller than could have been observed in the epidemiologic study. An upper limit of the cancer incidence is then calculated, assuming that the true incidence is just below the level of detection in the cohort studies. With this approach, the response is measured in terms of excess risk of the exposed cohort of individuals compared to the control group. In analyzing the data, it is assumed that the excess risk is proportional to the lifetime average exposure and that it is the same for all ages.

Both cancer epidemiologic data from occupational exposure to the carcinogen and cigarette smoking data are consistent with the multistage model for carcinogenesis (Day and Brown, 1980).

In view of current knowledge on the mechanism of carcinogenesis and human cancer epidemiologic observations it indicates the multistage model is the most appropriate model to use for the risk assessment of environmental carcinogens.

DISCUSSION

DR. MYRON MEHLMAN

Insufficient information was presented about assessing the biological effects of chemical mixtures. Moreover, the discussion of risk assessment and levels of exposure was based on too many assumptions. This entire area is in need of reexamination.

DR. ROBERT NEAL

An important concept in calculating cancer risk at low levels of exposure in man is the dose of the active compound or metabolite at the target site. The mathematical models currently used assume, in extrapolating from high-dose effects in experimental animals to potential low-dose effects in man, that the concentration of the active compound or metabolite at the target site changes linearly with exposure concentration. This is not necessarily true. In fact, there is a substantial body of data which suggests that the dose of the active compound at the target site will not decrease in a linear fashion with decreasing exposure concentrations. Thus more work needs to be done to determine the relationship between exposure dose and dose of the active compound at the target site. When we have knowledge of parameters such as this, we will be in the best position to modify our mathematical models to more accurately estimate potential cancer risk in man from low-dose exposure using data generated in experimental animals exposed to high levels of these compounds.

DR. WILLIAM NICHOLSON

I do not support any use of a safety factor approach for risk extrapolation of carcinogens, either for lifetime or short-term exposures. Although such extrapolations may give results similar to the standard carcinogen risk procedures in some cases, it is not convincing at all that such would be the general case. We have reasonable models for extrapolating carcinogenic risk to lower doses, both for chronic and short-term exposures. Their use should be continued.

The Use of a Threshold Model for Epigenetic (Nongenotoxic) Carcinogens

I strongly oppose the proposal discussed by Dr. Albert to utilize different models for genotoxic (as manifested through *in vitro* mutagenic test systems) and epigenetic carcinogens. My objections are as follows:

1. There are no data to indicate that thresholds generally exist for epigenetic carcinogens. In particular, one of the most studied of such materials, asbestos, demonstrates strong linearity of response over the entire range of available data. While it may be possible to construe certain situations in which metabolic deactivation, etc., can apply, one has no justification for assuming a threshold without the complete knowledge of how such processes operate in all potentially exposed individuals.
2. The use of mutagenic test systems to determine what is or is not a genotoxic carcinogen has limitations. Since many genotoxic carcinogens require activation, the choice of a proper test system is limited and accurate identification of these substances is difficult. A negative test may simply be an inadequate test.
3. Even if (as discussed by many authors) a threshold did exist for a certain carcinogen in the absence of exposure to any other similarly acting materials, other carcinogenic agents in the environment would influence health effects in humans. One is not adding an agent to an unexposed individual but to an individual already assaulted by many other initiators and promoters. In fact, in such circumstances a linear dose-response function, as determined by the extrapolation of the dose-

response relationship to zero dose, may not be conservative. Consider the dose-response relationship illustrated in Figure 24. Extrapolation from point A to the origin would produce a slope one-third less than the slope of a slight change along the curve at point B.

I would suggest that our information on the appropriateness of a linear dose-response relationship for genotoxic compounds is not better than that for the epigenetic ones. Until we have explicit information that would distinguish effects for each of these compounds, Dr. Albert's discussion on how to treat genotoxic agents would appear to be appropriate for all agents determined to be carcinogenic.

DR. REVA RUBENSTEIN

The discussion concerning promotion versus initiation highlighted still another difficulty. There was a definite sense of urgency about changing the risk assessment procedure, however sufficient data have not yet been developed. If we were better able to define epigenetic, we might be capable of investigating it. I heartily support such research, but I am adamantly opposed to change in the carcinogenic risk assessment procedures until the fruits of the research can be examined.

MR. WILLIAM GULLEDGE

Distinction Between Genotoxic and Epigenetic Carcinogens

Discussion by Dr. Albert on this subject seemed to indicate that the concept has received little consideration by the Carcinogen Assessment Group. This is unfortunate, as the focus of the two previous meetings and the informal poll taken by Dr. Albert at this meeting (~70% favor the distinction) clearly indicate acceptance and technical validity to a threshold mechanism. Support should be given for proposals already developed for separate risk assessment approaches for genotoxic and nongenotoxic carcinogens.

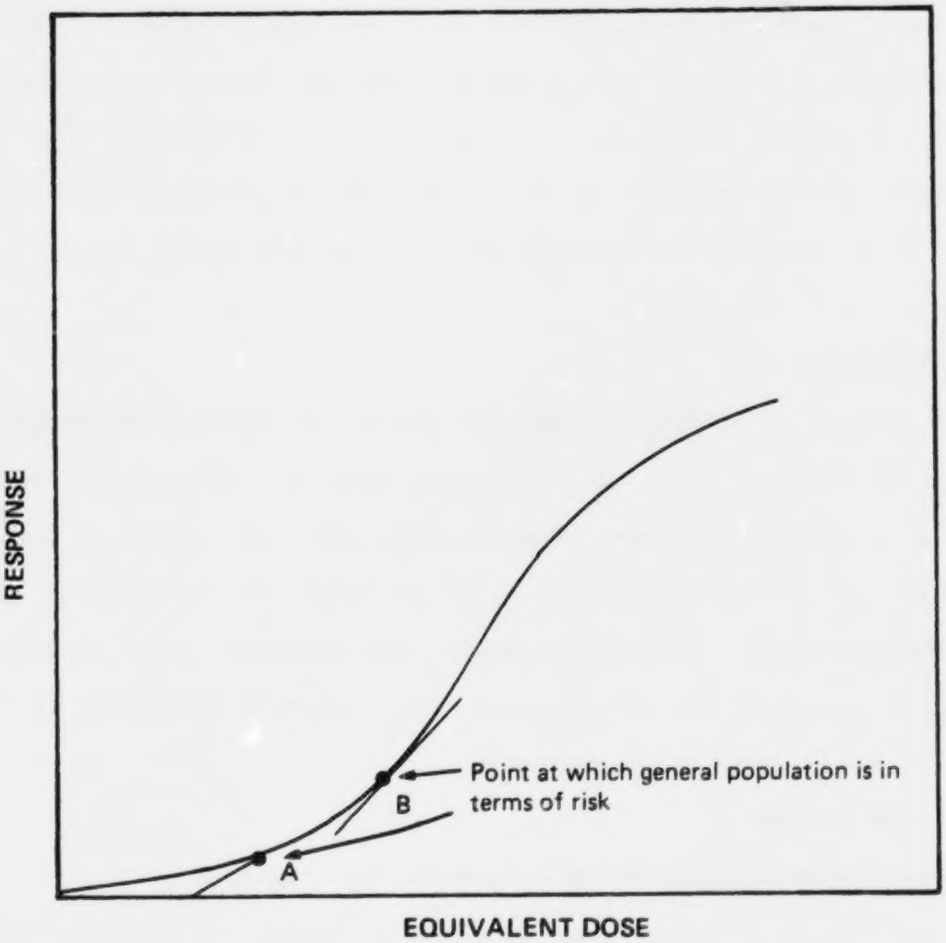


FIGURE 24

Hypothetical Dose-Response Relationship for Exposures to Multiple Agents

For epigenetic mechanisms, a mathematic model can be used to predict a 10^{-2} risk in humans from experimental animal data. Additional safety factors (taking into account negative data) can be applied as needed to account for human equivalent dose and various uncertainty factors associated with the adequacy of the data and extrapolating from average to sensitive individuals. Total risk factor would be approximately 10^{-5}, but would vary for each specific case. Policy, economic and other considerations should also be included in the overall decision, and this will be discussed separately.

DR. ROLF HARTUNG

The basic concepts of genotoxic vs. epigenetic causation of cancers still require clarification. Both processes produce somatic mutations which eventually express themselves as malignant tumors. Once a tumor has been produced, there may be no way of determining the mechanism by which it originated. The differences in the genotoxic and epigenetic causations of cancers are purely in their postulated mechanisms of origin. Genotoxic mechanisms are those which cause a direct alteration of the cellular DNA, by mechanisms such as direct alkylation or intercalation between adjacent nucleotides. The interactions are compatible with single-hit mechanisms, which are then modified by the normal states in tumor growth and/or suppression toward the final expression of a noticeable tumor by quantitative processes which can be expressed in terms of multistage dynamics.

In the case of epigenetic causations, a number of other phenomena predominate. The chemical cannot directly interact with the DNA, but causes alterations in the cellular DNA only after a series of intervening steps. One scenario that has been suggested is that the chemical or its metabolite causes severe cellular damage and necrosis, engendering extensive and repetitive repair over the lifetime duration of the exposure. This process forces an excessive number of cellular replications, many more than would be expected to occur during the course of a normal lifespan. Such a forced excessive cellular replication should result in a proportionately (or greater than proportional) increased number of transcription errors, some of which could have a carcinogenic outcome. In another scenario, a generalized enzyme inhibitor (e.g., a sulfhydryl reagent) would inhibit many enzymes throughout the body, including some of the enzymes involved in the synthesis and transcription of nucleotides to functional DNA. When some of the required enzymes are partially inhibited, it is very conceivable that the transcription of the DNA during cellular replication should become more error prone. Such a hypothesis may explain, in part, some of the events leading to heavy metal carcinogenesis. It should be noted that all the processes that I have invoked for the production of epigenetic effects require mass action, and some require enzyme kinetics or the overwhelming of normal functional reserve or repair mechanisms. Such phenomena are, at a minimum, more compatible with non-linear dynamics, and probably are also more compatible with threshold dynamics (it takes conceptually more than one molecule to do the dirty deed).

When viewed in this manner, it becomes apparent that one cannot always distinguish between genotoxic and epigenetic carcinogens on the basis of mutagenicity, because an agent may be mutagenic on the basis that it was able to inhibit many of the enzymes required for cellular replication.

I would suggest that the evaluation of the probable genotoxic or epigenetic causation of carcinogenicity of a chemical be based not on a check-list for critical tests, but on a coherent evaluation, which seeks to fit a plausible hypothesis to the compendium of experimental results in a way that makes sense. Since it is possible that some agents may act both through genotoxic and epigenetic mechanisms, one must take great care in the interpretation of the experimental results. Such interpretations cannot be done by rote, but to avoid them may result in severe and unnecessary harm to the economic fabric of the nation.

Let's spend more time on the basic issues of what is the meaning of epigenetic vs. genotoxic carcinogenicity in a regulatory context.

DR. RICHARD KOCIBA

There was strong support by the participants to have EPA and CAG reevaluate their present method of categorically doing mathematical risk assessment on all carcinogens by using the linearized multistage model to calculate an upper bound of carcinogenic potency. There is little justifiable scientific basis for continuing to use this previous approach to categorically deal with all carcinogens.

Extensive research has lead to significant advances in the understanding of the various mechanisms by which a carcinogenic response can be produced in laboratory animals. This research has also generated a more appropriate data base for extrapolation of results of cancer studies in laboratory animals to man. Recent reviews by Weisburger and Williams (1980, 1981) and Stott et al. (1981) have summarized some of the significant new developments resulting from this research.

It has become increasingly evident that all chemical carcinogens do not act via the same mechanism. Based on the extent of a chemical's interaction with DNA, it appears that chemicals that have a greater propensity to directly interact with DNA are appropriately classified as genotoxic. Those that do not have this propensity to interact directly with DNA, but lead to tumors via recurrent tissue injury or other secondary events are classified as nongenetic or epigenetic carcinogens. The carcinogenic risk to man posed by such epigenetic carcinogens appears to be substantially less that that posed by purely genotoxic carcinogens.

Certain of the chemicals categorized as carcinogens have been extensively studied with regard to their mechanisms of action and/or their comparative metabolism in man and animals. This is typified by the extensive data published by Schumann et al. (1980) indicating a nongenetic mechanism of carcinogenesis for tetrachloroethylene in the mouse. The work of Reitz et al. (1980) described a nongenetic mechanism of carcinogenesis for chloroform in the mouse and also concluded that man would be much less sensitive than the mouse or rat to chloroform. These advances in the understanding of the mechanisms of animal carcinogenesis and the development of a more appropriate basis for extrapolation to man has led to a critical reevaluation of the previous assumptions that have been used as the basis for regulatory action on materials categorized as carcinogens. Recent and significant developments in this respect include the following:

1. Dr. A. Kolbye, who has served as the Associate Bureau Director of Toxicology for the U.S. Food and Drug Administration (FDA), 1981, has repeatedly stressed the need to go beyond the previous simplistic categorization of chemicals into groups that do or do not cause cancer. Dr. Kolbye recommends that carcinogens be considered on the basis of their mechanism of action whereby they act (a) as a complete carcinogen via initiation by interaction with DNA, or (b) via secondary effects mediated by recurrent tissue injury, decreasing biological resistance, etc. Dr. Kolbye stated that the latter type "should not be called carcinogens", even though under some circumstances and at some doses they can influence cancer induction.

2. The Administrative Conference of the United States recently published a notice on carcinogenic regulation in the Federal Register (47 FR 11024, March 15, 1982). This notice pertains to recommendations addressed primarily to U.S. Federal Agencies that regulate carcinogens. Recommendations for the future include the use of peer review and advisory panels to address specific chemicals on a case-by-case basis in which all the relevant data and expertise can be used in the evaluation of possible carcinogenic risk to man.
3. Dr. R. Squire, who has served as the Director of the U.S. National Cancer Institute's program for the testing in animals of chemicals for carcinogenic potential has recently published (Squire, 1981) a series of recommendations on the appropriate interpretation and extrapolation of animal cancer studies to man. Dr. Squire noted that the nature and extent of data indicating carcinogenic effects in laboratory animals varies widely, yet present regulatory policy does not permit adequate discrimination among the many animal carcinogens. He pointed out the need for a case-by-case consideration that would allow a ranking of animal carcinogens based on the number of animal species affected, the types of different tumors induced, the spontaneous incidence rate of the tumors induced, the dose-response relationship, and the mechanism (genotoxic vs. epigenetic) by which the carcinogenic response occurred.
4. The Technical Committee of the Society of Toxicology (1981) recently examined the issue of regulation of potential carcinogens and stated that the assessment of human risk of carcinogenesis approaches credibility only when the material is examined under conditions "that reasonably approach human use and metabolic handling." The technical committee

also recommended a return to the use of the pragmatic approach to safety assessment that has historically proved so useful in the extrapolation of other animal toxicity data to humans.

5. The Council on Scientific Affairs of the American Medical Association (AMA, 1981) has recently criticized the basis of the past federal carcinogen regulation initiative. The Council stated that the previous underlying premises need to be revised or refuted, especially in view of "evidence both from experimental animals and man that there is a threshold for many carcinogens; thus, the concept of the threshold level beneath which exposure is harmless cannot be rejected."

6. On February 17, 1982, the Environmental Criteria and Assessment Office of the U.S. EPA held a "Workshop on Estimating Ambient Water Quality Criteria for Epigenetic Carcinogens". The objective of this workshop was spelled out in the workshop report (U.S. EPA, 1982) as follows:

> Prior to 1981, it was commonly held that somatic gene mutation was the principal mechanism by which compounds exerted carcinogenic effects. Somatic cell gene mutation is thought to show a one-to-one correspondence between dose and effect and is held to have no threshold. Regulatory agencies, espousing this belief, adopted a very protective stance toward health and therefore decreed that all carcinogens should be treated as genotoxins, as though their dose-response relationships were linear and without threshold. On this basis, methods were evolved for determining ambient water quality criteria. In the case of carcinogens, models used to extrapolate from the high doses used in animal experiments to the low doses, thought to be encountered by humans, incorporated this linear non-threshold concept. Criteria derived using such models in some cases were below ambient atmospheric levels, thus resulting in considerable concern, not only because they seemed unreasonable, but because the validity of the models in other situations might be questioned. More recently, other mechanisms of cancer have been suggested. These mechanisms are thought not to involve direct action of the chemical with the DNA and have thus been termed epigenetic. Epigenetic mechanisms, such as inhibition of DNA synthesis or repair enzymes, are thought to have threshold dose-response relationships. Therefore, it becomes necessary to develop new methods for estimating ambient water quality criteria for carcinogens with epigenetic (threshold) mechanisms.

As indicated by the objectives of that workshop, this is a significant development that must be pursued by EPA in order to utilize the newer data becoming available on this issue.

7. The regulatory agencies in the Netherlands (Kroes, 1982) have already incorporated changes in their carcinogen policy that distinguish between genotoxic and nongenotoxic (epigenetic) mechanisms of action. For genotoxic carcinogens, the more stringent nonthreshold (one-hit) model is used, but for nongenotoxic carcinogens, a less stringent extrapolation to man utilizes the application of a safety factor to the animal data.

As indicated by the numerous recent developments within regulatory and academic circles above, it is obvious that the significant new scientific information on carcinogenic mechanisms and extrapolation should lead to a broad-scale reassessment of the methodology used in the establishment of appropriate limits of human exposure for chemicals identified as carcinogens. This is especially true for those carcinogens acting via a nongenetic mechanism. This will undoubtedly lead to a more scientifically valid and realistic differentiation between those chemicals that truly warrant a more stringent degree of control of potential exposure compared to those chemicals that do not require the same stringent control of potential exposure.

Another critical decision point in the risk assessment process is the conversion of animal exposure data to human exposure data. In the past, it has been assumed that man is more sensitive than laboratory animals on a mg/kg basis because of his lower basic metabolic rates. This added conversion factor may be appropriate in some instances, but is not appropriate if the available data show that a metabolite or reactive intermediate is the proximate carcinogen rather than the parent compound. In these cases the body burden or blood concentration of the proximate carcinogen should be less for man than for laboratory animals because of his lower rate of metabolic processes.

DR. HARRY SKALSKY

When Dr. Albert asked for a show of hands, over 75% of the scientists present indicated that they supported the subdivision of carcinogens into genotoxic and nongenotoxic categories. There was strong support by the participants to have EPA and CAG reevaluate their present policy of categorically doing mathematical risk assessment on all carcinogens using the multistage model to calculate an upper bound of carcinogenic risk. Human risk assessment is an uncertain science, and it may well remain a matter of serious dispute for years. Nevertheless, guidelines are needed for the regulatory decision-making process. Those guidelines must be flexible to accomodate improvement mandated by an ever-advancing health technology. The Cancer Assessment Group's dogmatic use of a single model can in no way be considered flexible.

I understand that the IARC method of classifying carcinogens is currently being considered by EPA. It would appear that the adoption of the IARC classification would entail the use of different safety assessment procedures for each category. The "Carcinogen Policy" developed by the Committee of the Health Council of the Netherlands (1980) for consideration by CAG contains the flexibility that the CAG approach is missing. The Netherlands Health Council (1) has established different classes of carcinogens based on available scientific information (nongenotoxic, etc.); (2) has resisted a prior choice of a single extrapolation model, but will review a range of models; (3) suggests that a no-effect approach to safety assessment (NOEL-safety factor) may be appropriate for IARC carcinogen categories II-IV.

It would appear advantageous for CAG to develop a flexible policy that would accommodate new scientific concepts of chemical carcinogenesis.

In re-evaluating their policy, the CAG and the EPA should also consider a definition of what constitutes sufficient data for a risk assessment. As a scientist, I cannot believe that a two-dose NCI bioassay provides sufficient scientific evidence to predict the potential carcinogenic risk in man at 10^{-5}, 10^{-6} or 10^{-7}. It appears that experts have difficulty agreeing to what constitutes sufficient data for risk assessment when only human data is involved. In discussing the recent IARC monograph on benzene (IARC, 1982), Dr. Tomatis (1982) states:

> On pages 395 and 396 (of the monograph), there is a complete and objective summary of the available evidence of risks derived from exposure to benzene. It is clearly indicated that at 100 ppm the estimated relative risk for leukemia is increased more than 20-fold. Risks of this magnitude should attract attention to the possibility of significant risks at much lower levels. The IARC felt, however, that the data were insufficient to quantify precisely risks at lower levels.

There appears to be a disagreement between the IARC and the Cancer Assessment Group, for CAG has produced risk estimates for benzene in air and water and, evidently, believes the data are sufficient. In light of the $ED_{.01}$ Study and the differences in scientific opinions, the Cancer Assessment Group might want to formally review "what constitutes sufficient data for risk assessment." My ideas on risk assessment models, etc., are included as the second half of this comment.

The workshop participants emphasized a need to re-evaluate the procedures used to convert exposures in animals to exposure to man. This is especially true of the parameters utilized with CAG's multistage model. In the past, it has been assumed that man is more sensitive than laboratory animals on a mg/kg basis because of his lower basic metabolic rate. Certainly, there are exceptions to this as there are any other generalities.

The success of the physiologically-based pharmacokinetic models in pharmacology indicates that they may become a useful tool to toxicologists. Anderson (1981) has stated:

> Some of the predictions of the pharmacokinetic analyses are still tentative and require more definitive experimentation. Nonetheless, studies on saturable clearance of a variety of chemicals are causing a healthy reevaluation of several basic tenets of toxicology. These fundamental aspects include proper indexing of dose-response curves and better definition of what constitutes a biologically significant dose. This research is providing a much more in-depth appreciation of the complex interactions between biochemical and physiological variables which together control the rate of delivery and the rate of metabolism of ingested or inhaled chemicals *in vivo*.

The CAG should formally review these models to determine which internal parameters might be significant to relate to observed dose-response curves of carciongens. Such a review would provide needed information as to what specific data should be collected in future animal bioassays.

Quantitative Risk Assessments -- A Choice of Models

Human risk assessment is an uncertain science, and it may well remain a matter of serious dispute for years. Nevertheless, guidelines are needed for the regulatory decision-making process, even in the face of scientific uncertainties. Those guidelines must be flexible to accommodate improvements mandated by an ever-advancing health technology.

The process of risk extrapolation can be simply viewed as a procedure of predicting from known data what might be expected to occur in a region where there is no data. In dealing with potential carcinogens, the evaluation of risk is often complex. Complications arise due, primarily, to four factors. First, the majority of cancer data is acquired through laboratory experiments with rodents, not primates or human populations. Second, the amount of material administered to experimentally induce cancer is usually high.

Third, the incidence of cancer has to be high in order to discriminate it from control populations. Fourth, present scientific data does not clearly provide a definitive answer to the issue of a threshold for carcinogenesis. Thus, it is uncertain how to precisely utilize the known data and the mathematical direction of the extrapolation line becomes a matter of belief rather than scientific fact. Many theoretical dose-response models for the prediction of risk or carcinogenesis have been proposed (Mantel and Bryan, 1961; Armitage and Doll, 1961; Druckrey, 1967; Hoel et al., 1975; Guess et al., 1977; Cornfield, 1977; Albert and Altshuler, 1973; Chand and Hoel, 1974; Hartley and Sielkin, 1977; Crump et al., 1977; Reitz et al., 1978; Gehring and Blau, 1977; Mantel et al., 1975).

Van Ryzin (1980) recommends a use of a variety of models to get answers in or near the experimental range. Munro and Krewski (1981) provide a more complete analysis of risk assessment and regulatory decisions. They point out that:

> "Because of the uncertainties involved in assessing risks of low levels of exposure, some regulatory authorities have advocated the use of conservative risk assessment procedures [Interagency Regulatory Liaison Group (IRLG), 1979; U.S. EPA, 1980]. While linear extrapolation may be appropriate for potent electrophilic carcinogens, the use of such conservative procedures for less potent substances, which may induce tumors through perturbation of normal physiology, may not be warranted. In the latter case, however, the most suitable model for extrapolation is not at all clear."

Munro and Krewski warn that the quantification of human risk on the basis of the results of laboratory studies in animals should be approached with great caution. They advise that animal studies serve primarily as a qualitative surrogate for humans and that any attempts to quantify responses beyond the realm of biological certainty are open to serious question.

The wide variety of mathematical models available for risk assessment and the divergent answers they generate is confusing and the act of choosing one model over another will always stir controversy. However, there have been some unifying developments in this quandry, which would be worthy of consideration.

The $ED_{.01}$ study has demonstrated that all models tend to be equally predictive through a risk of 10^{-2}. After this point, the models begin to diverge drastically and it is clear that the biological relevance is lost. Since predictions beyond 10^{-2} are scientifically suspect, perhaps a combination of a mathematical model and safety factor approach should be considered rather than choosing a traditional risk model.

A model(s) could be used to predict a risk from a biological data set(s) to 10^{-2}, then a series of safety factors could be assigned to allow for consideration of the total data base (particularly negative data) and to clearly define areas where policy decisions have an overriding influence. Such an approach would be advantageous because the issues of science and policy would be clearly separated.

The mathematic model could be used to predict risk from biological data to the 10^{-2} limit. Then, a safety factor, or a combination of safety factors, could be used to consider the total data base, including negative data. This mathematic model safety factor method would also distinguish the scientific elements of setting permissible exposure levels from the elements based upon policy decisions. An example of such a quantitative risk model is described below:

Nongenotoxic carcinogens: These are threshold toxins and traditional approaches can be used.

$$\text{Acceptable Human Exposure (mg/day)} = \frac{\text{NOAEL (mg/kg/day)} \times 70\ \text{kg}}{100}$$

NOAEL = No-observed-adverse-effect level in experimental animals

70 kg = assumed average human weight

100 = 100-fold uncertainty factor which represents 10 for extrapolating from the average animal to the average human and 10 for extrapolating from the average to the sensitive human.

Genotoxic carcinogen: Model is devised to accommodate genotoxic carcinogens and compounds where genotoxicity has not been well defined by experimental evidence.

$$\text{Acceptable Human Exposure (mg/day)} \quad \frac{\text{Model } (10^{-2}) \quad \times \quad \text{Human Equivalent dose}}{10^{1} \quad \times \quad 10^{2} \ldots}$$

Approximate Risk Level

10^{-2} Model: Mathematical model (probit, multihit, multistage, etc.) utilized to predict risk to 10^{-2} from experimental animal data. (Used to predict only what would be expected to occur in rats, mice, etc.) The use of the 95% confidence interval of these models is equivalent to an additional safety factor of 2- to 5-fold, i.e., for an experiment of 10 animals ≃ 5, for 100 animals ≃ 3, 1000 ≃ 1.8. (Unless there is compelling evidence to the contrary, the 95% confidence interval should not be used. Where necessary it should be replaced with appropriate safety factors.)

10^{-1} Human Equivalent Dose: Model commonly used, involves:

$$\left(\frac{\text{length of exposure}}{\text{length of observation}}\right)\left(\frac{\text{length of observation}}{\text{lifespan of animal}}\right)^{3}$$

$$\sqrt[3]{\frac{\text{70 kg (average man)}}{\text{average animal weight}}}$$

This is a conservative model and should be modified where pharmacokinetic data are available. When no comparative metabolic data are available, this model will yield a safety factor of ~5- to 6-fold in the conversion of rat data to a human equivalent dose and ~13-fold for the conversion of mouse data.

10^{-1} 10_1 = uncertainty factor for extrapolating from average to sensitive man.

10^{-2} 10_2 = uncertainty factor associated with data base. With good negative data in other experimental animals, a factor of 1 might be used.

TOTAL RISK
FACTOR ____

10^{-5} 10 . . . -- policy and other parameters

This example deals in a preliminary fashion with data from experimental animals. If thoroughly developed, it would represent a way to separate and define issues of policy and science, which cannot be accomplished with other commonly used risk models. It would also provide the flexibility to accommodate the growth of scientific evidence and to utilize negative data.

DR. MARVIN SCHNEIDERMAN

Figure 25 shows some data relating to threshold. The data relate to some experiments in the 1950s by Walter Heston of NCI (Heston and Schneiderman, 1953; Mantel et al., 1961). He was interested in the somatic mutation theory of cancer and was concerned whether he needed one or two mutations to produce the lung tumors in Strain A mice. Heston postulated that a single mutation would lead to a straight-line dose-response; two mutations would lead to a quadratic dose-response curve. Figure 25 shows what he found in a series of experiments. The first experiment produced a nice straight line displaced to the right. This would be consistent with a one-mutation process with a threshold. Because of previous work by Charles and Luce-Clausen (1942), Dr. Heston questioned this apparent threshold for the tumor system and the chemical (2,4,5,6-dibenzanthracene) he was working with. (He did estimate the "threshold" dose, however.) So he conducted another series of experiments -- with doses down in the "threshold" range -- doses lower than those he had employed before. Result? As shown in Figure 25: a

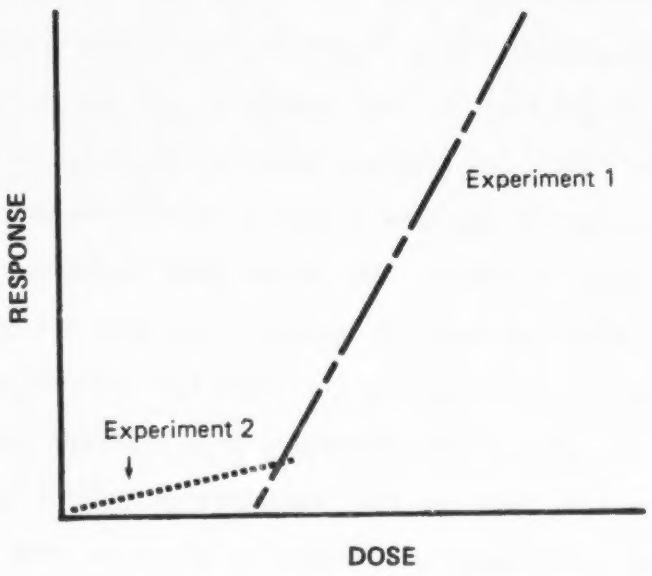


FIGURE 25

Dose-Response Data in Two Experiments Producing Lung Tumors in Strain A Mice

Source: Heston and Schneiderman, 1953

shallow dose-response curve in the low-dose region. Probably no threshold. Possibly a two- or even three-stage process, with "background" dose contributing a small amount to the number of lung tumors found in these animals at these low doses.

These observations bring us back to the concepts of dose additivity and models of carcinogenesis. Crump et al. (1976) showed that with the multistage model, an assumption of dose-additivity at low doses leads directly to the linear, non-threshold model. This is true for every stage in the multistage model of carcinogenesis. Thus linearity, non-threshold depends not on whether a material is genotoxic or not genotoxic, as some research workers have suggested, but rather on whether there is anything in the ambient environment that operates in the same manner in the carcinogenic process as the toxic material under question. The major thing to notice is that the later the stage at which the material operates, the more likely it is that reduced (or eliminated) exposure to the material will produce earlier response, and with it, more of the appearance of a possible threshold. The take-away lesson for both research and regulatory priorities would seem to be: find late-stage carcinogens and reduce exposures to them drastically. This should produce early reduction in cancers. For long-term reduction in cancer, restricted exposure to early stage (and "complete") carcinogens should produce the most effect.

DR. IAN NISBET

With one exception (see paragraph below), I am strongly opposed to proposals to use separate methods of risk assessment for "genotoxic" and "nongenotoxic" carcinogens, even if these can be operationally defined. The most important reason for assuming that dose-response relationships are linear and non-threshold at low doses is the principle of dose additivity.

The proof that dose additivity leads to linear nonthreshold dose-response relationships is a very general one (Crump et al., 1976) and applies in all cases except where the underlying dose-response relationship is of a strict threshold type and the background incidence is exactly zero. In accordance with this, the multistage model predicts linear dose-response relationships for chemicals acting at all stages. In my opinion, the burden of proof should be on those who believe that late stage carcinogens have thresholds to prove that their effects are not additive to background. It should be noted that the human population has a background incidence of all the phenomena that are hypothesized to be modes of action of "nongenotoxic" carcinogens, including metabolic overload and gross tissue damage. This is perhaps a bias that comes with middle age, but I think that late-stage carcinogens are of greater concern than early-stage carcinogens.

One real difference between early- and late-stage carcinogens is in the rate of decline of excess risks after cessation of exposure (Day and Brown, 1980). I think that risk assessments should take into account information on the stage of action if the exposure assessment indicates that exposure will cease substantially before the end of life.

DR. JAMES WITHEY

We are still "up in the air" with respect to the question of what to do with mutagens. My own feeling is that the air has become cloudy as a consequence of the increasing complexity of these tests and the arguments about genotoxics, promoters, initiators, etc. Right now I think that mutagenicity might be used as an indicator of potential carcinogenicity and to support carcinogenicity data.

REFERENCES

Albert, R.E. and B. Altshuler. 1973. Considertions relating to the formulation of limits for unavoidable population exposures to environmental carcinogens. AEZ Symposium Series. Conference 72050. NTIS. p. 233-235.

AMA (American Medical Association). 1981. Carcinogen regulations. Report of the Council on Scientific Affairs. J. Am. Med. Assoc. 246: 253.

Ames, B.N., J. McCann and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with Salmonella/mammalian microsome mutagenicity test. Mutat. Res. 31: 347.

Anderson, M.E. 1981. Saturable metabolism and its relationship to toxicity. CRC Crit. Rev. Tox. p. 105-149, May.

Armitage, P. and R. Doll. 1961. Stochastic models for carcinogenesis. In: Proc. of the 4th Berkeley Symposium of Mathematical Statistics and Probability: Biology and Problems of Health. 4: 19.

Chand, N. and D.G. Hoel. 1974. A comparison of models for determining "safe" levels of environmental agents. Reliability and Biometry. Soc. Ind. Appl. Math. p. 382-401.

Charles, D.R. and E.M. Luce-Clausen. 1942. Cancer Res. 2: 261.

Cleaver, J.E. and D. Bootsma. 1975. Xeroderma pigmentosum: Biochemical and genetic characteristics. Ann. Rev. Genet. 9: 19.

Committee of the Health Council of the Netherlands. 1980. The Evaluation of the Carcinogenicity of Chemical Substances. Government Printing Office, The Hague.

Cornfield, J. 1977. Carcinogenic risk assessment. Science. 198: 693-699.

Crump, K.S., D. Howell, C. Langley and R. Peto. 1976. Fundamental carcinogenic processes and their implications for low-dose risk assessment. Cancer Res. 36: 2973-2979.

Crump, K.S., H. Guess and K. Deal. 1977. Confidence intervals and test of hypotheses concerning dose-response relations inferred from animal carcinogenicity data. Biometrics. 33: 437-451.

Day, N.E. and C.C. Brown. 1980. Multistage models and primary prevention of cancer. J. Natl. Cancer Inst. 4: 977-989.

Diaz Gomez, M.I. and J.A. Castro. 1980. Covalent binding of carbon tetrachloride metabolites to liver nuclear DNA proteins and lipids. Toxicol. Appl. Pharmacol. 56: 199.

DiMayorca, G., M. Greenblatt, T. Frauthen, A. Soller and R. Giordono. 1973. Malignant transformation of BHK_{21} Clone 13 cells in vitro by nitrosamines -- a conditional state. Proc. Natl. Acad. Sci. USA. 70: 46.

Druckrey, H. 1967. Quantitative aspects of chemical carcinogenesis. In: Potential Carcinogenic Hazards from Drugs (Evaluation of Risk). U.I.C.C. Monograph Series, Volume 7. Springer-Verlag. p. 60.

Gehring, P.J. and G.E. Blau. 1977. Mechanisms of carcinogenesis: Dose response. J. Environ. Pathol. Toxicol. 1: 163-179.

Guess, H., R. Peto and K.S. Crump. 1977. Uncertainty estimates for low dose rate extrapolation of animal carcinogenic data. Cancer Res. 37: 3475-3483.

Hart, R.W., F.B. Daniel, O.R. Kindig, C.A. Beach, L.B. Joseph and R.C. Wells. 1980. Elemental modifications and polycyclic aromatic hydrocarbon metabolism in human fibroblasts. Environ. Health Perspect. 34: 59.

Hartley, H.O. and R.L. Sielken. 1977. Estimation of safe doses in carcinogenic experiments. Biometrics. 33: 1-30.

Heston, W.E. and M.A. Schneiderman. 1953. Analysis of dose-response relation to mechanisms of pulmonary tumor induction in mice. Science. 117: 109-111.

Hill, A. and S. Wolff. 1982. Increased induction of sister chromatid exchange by diethylstilbestrol in lymphocytes from pregnant and premenopausal women. Cancer Res. 42: 893.

Hoel, D.G., et al. 1975. Estimation of risk of irreversible delayed toxicity. J. Environ. Health. 1: 133-151.

IARC (International Agency for Research on Cancer). 1979. Monograph on the Evaluation of Carcinogenic Risk of Chemicals to Man. 20: 371.

IARC (International Agency for Research on Cancer). 1982. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Volume 29. Some Industrial Chemicals and Dyestuffs. IARC, WHO Publications Centre, Albany, NY.

IRLG (Interagency Regulatory Liaison Group). 1979. Scientific basis for identification of potential carcinogens and estimation of risk. J. Natl. Cancer Inst. 63: 241.

Kroes, R. 1982. Presentation on Carcinogen Policy in the Netherlands. Toxicology Forum, February 15.

Lee, J.E., R.B. Ciccarelli and K.W. Jennette. 1982. Solubilization of the carcinogen nickel subsulfide and its interaction with deoxyribonucleic acid and protein. Biochem. 21: 771.

Lutz, W.K. 1979. In vivo covalent binding of chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. Mutat. Res. 65: 289.

Mantel, N. and W.R. Bryan. 1961. Safety testing of carcinogenic agents. J. Natl. Cancer Inst. 27: 455-470.

Mantel, N., W.E. Heston and J.M. Gurian. 1961. Thresholds in linear dose-response models for carcinogenesis. J. Natl. Cancer Inst. 27(1): 203-215.

Mantel, N., N. Bohidar, C. Brown, J. Ciminera and J. Tukey. 1975. An improved "Mantel-Bryan" procedure for safety testing of carcinogens. Cancer Res. 35: 865-872.

Metzler, M. 1981. Studies on the mechanism of carcinogenicity of diethylstilbestrol: Role of metabolic activation. Food Cosmet. Toxicol. 19: 611.

Miller, J.A. 1970. Carcinogenesis by chemicals: An overview. G.H.A. Clowes Memorial Lecture. Cancer Res. 39: 559.

Munro, I.C. and D.R. Krewski. 1981. Risk assessment and regulatory decision-making. Food Cosmet. Toxicol. 19: 549.

NCI (National Cancer Institute). 1976. Report on Carcinogenesis Bioassay of Chloroform. NTIS PB-264018, Springfield, VA.

Perera, F.P. and I.B. Weinstein. 1982. Molecular epidemiology and carcinogen-DNA adduct detection: New approaches to studies of human cancer causation. J. Chron. Dis. 35: 581.

Pitot, H.C. and C. Heidelberger. 1963. Metabolic regulatory circuits and carcinogenesis. Cancer Res. 23: 1694.

Prehn, R.T. 1964. A clonal selection theory of chemical carcinogenesis. J. Natl. Cancer Inst. 32: 1.

Rajalakshmi, S., P.M. Rao and D.S.R. Sarma. 1982. Chemical carcinogenesis: Interactions of carcinogens with nucleic acids. In: Cancer -- A Comprehensive Treatise, 2nd ed., F.F. Becker, Ed. Plenum Press, NY. p. 335-409.

Reitz, R.H., P.J. Gehring and C.N. Park. 1978. Carcinogenic risk estimation for chloroform: An alternative to EPA's procedures. Food Cosmet. Toxicol. 16: 511-514.

Reitz, R., J. Quast, W. Stott, P. Watanabe and P. Gehring. 1980. Pharmacokinetics and macromolecular effects of chloroform in rats and mice: Implications for carcinogenic risk estimation. In: Water Chlorination: Environmental Impact and Health Effects, Vol. 3, R. Jolley, W. Brungs, R. Cumming and V. Jacobs, Ed. Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

Robison, S.H. and M. Costa. 1982. The induction of DNA strand breakage by nickel compounds in cultured chinese hamster ovary cells. Cancer Lett. 15: 35.

Rom, W.N., G.K. Livingston, K.R. Casey, et al. 1983. Sister chromatid exchange frequency in asbestos workers. J. Natl. Cancer Inst. 70: 45.

Schumann, A. J. Quast and P. Watanabe. 1980. The pharmacokinetics and macromolecular interactions of perchloroethylene in mice and rats as related to oncogenicity. Toxicol. Appl. Pharmacol. 55: 207.

Setlow, R.B. 1978. Repair deficient human disorders and cancer. Nature. 271: 713.

Sirover, M.A. and L.A. Loeb. 1976. Infidelity of DNA synthesis _in vitro_: Screening for potential metal mutagens or carcinogens. Science. 194: 1434.

Society of Toxicology. 1981. Criteria for human risk assessment: With special emphasis on the regulations of potential carcinogens. Fundamental Appl. Toxicol. 1: 2.

Squire, R. 1981. Ranking animal carcinogens: A proposed regulatory approach. Science. 214: 877-880.

Stott, W., R. Reitz, A. Schumann and P. Watanabe. 1981. Genetic and nongenetic events in neoplasia. Food Cosmet. Toxicol. 19: 587.

Tomatis, L. 1982. Letter to the journal. Science. 218: 214.

Ts'o, P.O.P. 1981. Neoplastic transformation, somatic mutation, and differentiation. _In_: Carcinogenesis, Fundamental Mechanisms and Environmental Effects, B. Pullman, P.O.P. Ts'o and H. Gelboin, Ed. D. Reidel Publ. Co., Boston. p. 297-310.

U.S. EPA. 1980. Water Quality Criteria Documents. Federal Register. 45: 79318.

U.S. EPA. 1982. Report on Workshop on Estimating Ambient Water Quality Criteria for Epigenetic Carcinogens. Washington, DC. February 17.

Van Ryzin, J. 1980. Quantitative risk assessment. J. Occup. Med. 22: 321.

Weisburger, L. and G. Williams. 1980. Chemical carcinogens. In: Toxicology: The Basis Science of Poisons, 2nd ed. J. Doull, C. Klaassen and M. Amdur, Ed. MacMillan Publishing Company, NY.

Weisburger, J. and C. Williams. 1981. Carcinogen testing: Current problems and new approaches. Science. 2 4: 401.

HEALTH ASSESSMENT OF EXPOSURES TO CHEMICAL MIXTURES

September 30, 1982

HEALTH ASSESSMENT OF EXPOSURES TO CHEMICAL MIXTURES

Outline of Issues and Review of Present Approaches

Presentation:	Dr. Jerry F. Stara ECAO, OHEA, U.S. EPA
Presentation:	Dr. Richard Hertzberg ECAO, OHEA, U.S. EPA

PRESENTATIONS

DR. JERRY STARA: OUTLINE OF ISSUES

Simultaneous exposure to several chemicals is a predominate occurrence in our environment. The Agency recognizes the need for specific guidelines to assess the impact on human health. However, it also recognizes that it may not be possible to develop a scientifically defensible methodology to resolve all the issues associated with this complex subject. One of the major purposes of the following series of presentations on "Health Assessment of Exposures to Chemical Mixtures" is to identify areas in which reasonable scientific judgments can be made and methodologic modifications can be proposed, as well as to identify those areas in which limitations in our understanding and knowledge preclude methodologic development.

DR. RICHARD HERTZBERG: REVIEW OF PRESENT APPROACHES

To date, about a half dozen Superfund-designated sites have been investigated. One or two marker chemicals have dominated the situation in some easily identified sites. For real world situations, the American Conference of Governmental Industrial Hygienists (ACGIH) graded response approach based on additivity of equitoxic doses is used:

$$I = \sum_{i=1}^{N} d_i/A_i$$

where d_i equals exposure and A_i equals "acceptable" level. When $I>1$, there is cause for concern. If this approach is used, the implications of various dose-response curves must be considered, and an estimate of the chance of significant interactions (e.g., synergism) must be made.

DISCUSSION

DR. MYRON MEHLMAN

Acute data, such as LD_{50}s and LC_{50}s, are not useful for extrapolating to the type of injury that may result from low-level chronic exposure. The acute data are useful for labeling substances for transportation and for developing exposure levels for repeat dose exposure. What is probably needed is a methodology that will yield data on chemical mixtures by inhalation and gavage which will reflect actual exposure to man.

The approaches to this could be:

1. Gather information on:
    a. Exposure
        - levels
        - extent
        - frequency
    b. Chemical properties
        - group chemicals according to structure
        - select representative chemicals from structural classes (analogues)
    c. Reports in literature
    d. Structure-activity relationships
2. Determine additional information needed
3. Set up testing programs to develop this information
    a. Nongenetic
        - reproductive (short-term tests; male, female)
        - teratologic (short-term tests)
        - subchronic (pathology, bioaccumulation)
        - pharmacologic (blood levels, target organs, metabolites, half-life)

b. Genetic

- *In vitro* (with individual components)

    Ames
    Mouse lymphoma
    DNA repair
    Cytogenetics

- *In vivo*

    Assay urine for water-soluble compounds and feces for non polars from 30-day study in Ames and mouse lymphoma tests

4. Develop pharmacologic models
5. Using this data, develop a mathematic model to estimate risk
6. Perform professional biologic assessment to set exposure level

DR. MARVIN LEGATOR

Risk Estimates: The Non-Science

As to both systemic toxicity and carcinogenicity, I have become increasingly less comfortable by the derivation of specific numbers based either on safety factors or mathematical models. The scientific basis for adding safety factors is totally lacking, and mathematical modeling does not help in resolving the multiplicity of uncertainty factors when extrapolating animal data to man. In either case, I am afraid we come up with an almost meaningless number. Although, as described in several papers in this meeting, all sorts of caveats are given by scientists when presenting a specific numerical risk figure, the final figure is usually labeled as a definitive indicator of human risk. It may be that for water quality we need to derive a numerical estimate to set upper limits for specific chemicals. In the case of hazardous waste sites, perhaps we should abandon doing quantitative risk estimates. An alternative to deriving a specific figure may include the following elements:

1. Collect exposure data to indicate number and quantity of chemicals at a specific dump site (incorporate specific factors presented by Dr. Nisbet).
2. Determine how many chemicals are teratogens, carcinogens, systemic toxicants, etc.
3. For each chemical in each toxic category, use a ranking procedure to establish categories of concern. Possible models for this categorization are to be found in the Food Safety Council discussion on mutagenicity (Food Safety Council, 1978) and the Squire scheme for carcinogens (Squire, 1981). Rather than a specific number, we will use the animal data to reflect relative potency. Three or five categories can be established for each toxic class.

This type of ranking avoids the artifactual specific number and more accurately reflects the preciseness of translating data from one species to another. Taking into consideration the exposure data, the number of chemicals present and their specific potency, the hazard of a particular site can be determined. I would like to expand at a later date on this ranking approach for hazardous waste sites.

MR. WILLIAM GULLEDGE

Alternative Approaches in Risk Assessment

My comments are offered as an observation of human health-based and aquatic life-based water quality criteria development and possible application to hazardous waste disposal site modification. Human health-based criteria development uses compound-specific approaches which rely on quantitative chemical speciation and quantitative risk assessment modes. Aquatic life-based criteria development is based on a generic toxicity approach that uses generic bioassays to assess acute and chronic effects in aquatic organisms. It appears that there may be some technical and cost advantages to a generic toxicity approach, however its feasibility to application of human health criteria may be limited. Generic toxicity may be an important tool for hazardous waste disposal site risk assessment. It could be a very cost-effective technique for preliminary screening of hazard and the degree of hazard.

DR. REVA RUBENSTEIN

It would have been more fruitful to examine how much the outcomes change when the underlying assumptions are changed. We frequently are told that TLVs are established for the "mythical" 70 kg, 21-year-old male worker. How different will the numbers be if they are established for a 55 kg, 21-year-old female worker, and so on.

DR. MAGNUS PISCATOR

There was no discussion at all of some other problems, such as how to conduct studies at a waste site. The exposed people are probably not interested in extrapolated data from animal experiments. They want to know if they are sick or if there is a risk for birth defects etc. Thus good methods for studies of effects are needed. Is it possible to find good reference populations for an exposed group? This must be discussed some time.

Reference was made to the AGIH index for exposure to several chemicals, but it was pointed out that ths assumes exposure to a group of chemicals with similar properties (e.g., sme solvents).

DR. KURT ENSLEIN

Insofar as the estimation of health effects from multiple chemical exposures is concerned, I discused this matter at length with my group here in Rochester and we have come up with what may be an approach that could conceivably be useful. If you recall our structure-activity models, particularly the rat LD_{50} equation, you will remember that this equation consists of a large number of substructural fragments and molecular weight, each of these being assigned a weight. If one considers a mixture to be made up of nearly identical portions of separate chemicals, say two, then it is conceivable (and this was suggested by Dr. Clarkson) that one could simply add the appropriate substructural fragments to arrive at an estimate of the toxicity of the two chemicals in conjunction with one another, under the assumption that there will be neither synergism nor antagonism. As one now increases the number of chemicals, more and more of the fragments from the equation will be used, so that eventually all the fragments will be used to some extent. Under those circumstances we know that the asymptotic rat oral LD_{50} will be 1700 mg/kg (this number comes from the constant in our equation and is based on the 1851 chemicals from which the equation was calculated).

At the other end of the spectrum, suppose one chemical was dominant, or for that matter, that one chemical was much more toxic than the others at the particular site. Then one would be able to say that LD_{50} for that dump lies somewhere between that of the most toxic chemical, and is at worst 1700 mg/kg. In fact, if there are only a few chemicals in the dump, the estimation problem of course doesn't exist.

It would be interesting to experimentally check these ideas by testing appropriate combinations of chemicals in the rat oral LD_{50} assay. If such an experimental scheme were properly designed, we would also learn about the single or multiple weighting to be given to those fragments which appear in more than one chemical. It is also not too difficult to imagine various ways in which synergism and antagonism could be accounted for. The same general principles could also be applicable to the other endpoints that we have presently modeled, as well as other endpoints that would be modelable in the future. This is particularly the case for aquatic and inhalation toxicity.

DR. HARRY SKALSKY

Our discussions on the health assessment of exposures to chemical mixtures have underscored the complexities involved in attempting to evaluate multiple chemical exposures as they pertain to dump sites. It does not appear that there can be a "methodology" developed that will cover all the situations. Each dump site will entail its own unique problems, whether it be its proximity to populous areas, specific hydrology or the variety of chemicals it contains. There does not appear to be any substitute for a case-by-case approach. Perhaps the most compelling need surrounding the assessment of a particular site is the gathering of accurate exposure information. It would appear that the Agency would need to devise a procedure to identify and quantitate the individual chemical exposures at a dump site location. Without this exposure information, it is almost impossible to accurately assess potential hazard to a specific population. I would think a response team could be developed with a priority of gathering exposure data as the first step in reacting to notification of a potential dump site problem. Judgments concerning public safety could then be made on the basis of sound exposure data rather than a "hunch".

The identification of the chemicals involved and a quantification of exposures is a necessary first step. Then judgment as to the effect of these exposures, or presence of a hypersensitive population, etc., can be made on a case-by-case basis.

GENERAL COMMENTS

- The ACGIH graded response formula was intended to be applicable only to similar components (e.g., a mixture of solvents), not to a mixture of unrelated compounds. The similarity is assumed to apply to target organ, kinetics, and overall uptake. Application to mixtures of dissimilar compounds, assuming no synergism, would likely result in overestimating the actual toxicity. For carcinogens at low risk levels, the addition of risks is probably suitable.

REFERENCES

Food Safety Council. 1978. The proposed system for food safety assessment. Food Cosmet. Toxicol. 16(Supplement 2): 1-136.

Squire, R.A. 1981. Ranking animal carcinogens: A proposed regulatory approach. Science. 214: 877-880.

HEALTH ASSESSMENT OF EXPOSURES TO CHEMICAL MIXTURES

Assessment of Exposure

Presentation:	Dr. James Falco Exposure Assessment Group, U.S. EPA
Presentation:	Dr. Ian Nisbet Clement Associates

PRESENTATIONS

DR. JAMES FALCO: ASSESSMENT OF EXPOSURES

Introduction

Exposure assessments are critical to the evaluation of the potential public health risks due to exposure to toxic chemicals. Several components are needed to estimate the extent of the exposure:

1. An estimate of the releases into the environment, as well as monitored ambient concentrations.
2. An estimate of the number of people potentially exposed.
3. A description and quantification of characteristics of the exposed population.

Chemical-specific (e.g., fate and transport) and site-specific (e.g., ecological) data are also necessary components of complete exposure assessments. Each component will directly affect the accuracy and the resulting implications of each assessment.

Present Approach

The present approach used to estimate whole-body dose from ambient environmental concentrations is to treat complex mixtures as a set of independently acting chemical agents. The environmental concentration of each constituent is determined, and then the dose from each constituent is estimated as if it were acting singly.

Possible Approach

For complex mixture exposure assessments, the following estimates could be made:

1. Concurrent release rates.
2. Temporal and spatial variations in environmental concentrations.
3. Population exposed.
4. Exposures to each chemical and simultaneous exposure to two or more chemicals.

At least two major problems specific to complex mixtures must be overcome to make this approach workable. First, an estimation of the timing of releases must be made, and second, any modifications of environmental behavior due to chemical interactions must be understood.

DR. IAN NISBET: ASSESSMENT OF EXPOSURES

To make exposure assessments, one must take into account the behavior of the chemical in the environment and the behavior of the individual exposed. Environmental factors include release rates, environmental transport and ambient concentrations in various media, including considerations of space and time variations. Factors related to the exposed individual include both population characteristics (e.g., numbers, demography, movement patterns and susceptibility factors) and uptake factors (e.g., activity patterns, intake rates, absorption factors and pharmacokinetics). Large fluctuations in exposure rates produce the need to statistically express the frequency of exposure to different concentration levels and the time scale of the fluctuations. The best exposure assessments will use information from all of the following sources: ambient monitoring, models, target monitoring, analogies and surrogates.

Meaningful assessments of exposure must include assessment of temporal variability and duration. In multichemical situations, the constitution of the mixtures to which people are exposed varies in both time and space, so that no single measure of exposure can be adequate. I recommend the use of several indicator (or surrogate) chemicals, and toxicity testing of environmental mixtures to derive a relationship between risk and exposure to the indicator chemicals (including variability of this relationship). Recognizing the practical difficulties, I recommend using target monitoring (human tissues and sentinel animals, not wild animals) as an invaluable tool in exposure assessment.

DISCUSSION

DR. JULIAN ANDELMAN

In the presentation on assessment of exposure by Dr. Nisbet, as well as the critique by Dr. Piscator on subpopulations at greater risk (see the following chapter), the likelihood of encountering log-normal exposures and uptakes in an exposed population was appropriately raised. Thus, in addition to considering the increased sensitivity of certain segments of the exposed population, particular attention should be directed to the question of the variability of exposures and body burdens among groups that might nominally be expected to have the same exposure. Such variabilities can and do arise as a result of individual behavior, as well as varied physiology. This is graphically shown in geographically discrete populations of children who have log-normally distributed concentrations of lead in their blood. An example of the implications of this phenomenon is the assessment of the likely exceedance of a threshold level (e.g., NOAEL) via a given route of exposure, such as drinking water. Assuming the water concentration and a 2 ℓ water intake would correspond to this NOAEL, the fraction of the population likely to exhibit an effect might be considerably less than 100% due to the log-normal question. This could be addressed, however, through the uncertainty factor mechanism, but should be specifically considered as a principle to be incorporated in some fashion.

MR. WILLIAM GULLEDGE

Disadvantages of Transport Modeling Approach

Discussion at the workshop revealed the belief that transport modeling can frequently yield results which can be 1-2 orders of magnitude off measured results. This is certainly the case with respect to chemical fate modeling, which is a poor tool for exposure assessment. Very few, if any, models have been properly field validated. Laboratory validation, using microcosms or an alternative experiment, does not provide a true indicator for performance in the environment.

Another disadvantage of modeling is the extensive data requirements for input into the program. A typical model which could be used for water quality assessment and criteria development includes extensive input parameters for the water column and sediment interactions. Measurement for partitioning, hydrolysis, oxidation, biodegradation, photolysis and volatilization must be taken for the water column. Assessment of sedimentation, resuspension, density and solids concentration must be made for the sediment. These data must be obtained before most models are run, an expensive process, and the results used for human health exposure assessment.

Use of Surrogates for Multi-Chemical Exposure

Several presentations during the workshop alluded to the use of indicator or surrogate compounds for hazard assessment. Research in this area has shown poor correlation between selected surrogates and actual toxic compounds. In a study conducted by the Chemical Manufacturers Association (CMA), "no statistically reliable correlations were found between conventional and nonconventional pollutants and toxic organic pollutants." The indicator concept was applied to volatiles, base/neutral, and acid fractions with mixed results. Very few correlations were observed, with one exception being 2,4-dichlorophenol. Additional research is necessary.

DR. ROLF HARTUNG

The relationships of intermittent or fluctuating exposures to steady experimental exposures need further study, using more sophisticated analytical tools than Haber's Rule or Siderenko's modification.

DR. IAN NISBET

Most cases of injury by environmental chemicals have multiple causes. A consequence of the phenomenon of dose-additivity-with-background is that, at low doses of the environmental agent, all of the observed cases will be strongly associated with background causes, and only weakly associated with the incremental exposure to the agent. (For example, most of all asbestos-induced lung cancers occur in smokers.) Unless multiple causation is explicitly recognized, we will underestimate the effect of environmental agents. (This is what I would like to be known as the Doll-Peto fallacy).

GENERAL COMMENTS

- There is a problem in obtaining data from dump sites because of the need to sample quickly and decide what to do for all government bodies and citizen groups involved.
- Use of exposure models without real data produces a great deal of uncertainty.
- A simple statistical model assuming a log normal distribution of exposure is one of the most useful models.
- Target monitoring is probably the most useful method but unless there is a surrogate chemical to which there has been a high enough exposure, it will be difficult to plan an exposure assessment around target monitoring alone, due to matrix problems in analyzing very small sample size of the various chemicals. However, some guidance for future study design can be obtained from even one tissue sample.
- For chemicals that are not unique to the dump site, such as lead, contribution from other sources must be considered.
- There is a problem of double counting volatile compounds (e.g., exposure from water may be measured and then as the compound volatilizes, the air level is measured).
- Distribution of exposure has not been characterized for multichemical exposures.
- There is a working assumption to use log normal since it fits the data.
- The use of tracer chemicals is not being considered since it would take too long to monitor groundwater movement.
- Measurement of the toxicity of the unknown materials and their migration potential should be considered.

HEALTH ASSESSMENT OF EXPOSURES TO CHEMICAL MIXTURES

Subpopulations at Greater Risk

Presentation:	Dr. Linda Erdreich and Ms. Cynthia Sonich Mullin ECAO, OHEA, U.S. EPA
Critique:	Dr. James Withey Food Directorate, Bureau of Chemical Safety
Critique:	Dr. Eula Bingham University of Cincinnati
Critique:	Dr. Magnus Piscator University of Pittsburgh

PRESENTATION

DR. LINDA ERDREICH AND MS. CYNTHIA SONICH MULLIN: HYPERSUSCEPTIBLE SUBGROUPS OF THE POPULATION IN MULTICHEMICAL RISK ASSESSMENT

Introduction

The existence of hypersusceptible individuals has been recognized by the EPA, even in the absence of specific data on the response of hypersusceptible humans or experimental animals. However, even when specific subgroups have been identified, they are often only considered qualitatively in criteria derivation. Little consideration has been given toward the methodical protection of specific hypersusceptible subgroups. The goal of this presentation is to critically assess the need to identify and protect such individuals, particularly in the context of risk assessment following exposure to toxic waste sites. In this regard, two main issues will be addressed:

1. To what extent does the present approach protect high-risk, including hypersusceptible, subgroups of the population?
2. Do these hypersusceptible individuals comprise a proportion of the population that is sufficiently large to justify the systematic consideration of high-risk groups in the risk assessment process?

Prior to addressing these issues, specific terminology and background must be provided. Hypersusceptibility and sensitivity have been used interchangeably. Redmond (1981) defines sensitivity as "responsiveness to a pollutant", where sensitivity refers to the rate of change of a response as the dose increases. We prefer the use of the term hypersusceptibility in that it simply implies "more susceptible." A hypersusceptible individual is one who will experience an adverse health effect to one or more pollutants, significantly before the general population, because of one or more factors which predispose the individual to the harmful effects.

These individuals are essentially at higher risk of adverse health effects due to exposure to the pollutants. However, "high risk" is often used to designate specific groups, such as occupational groups, that are at higher risk because they are exposed to higher exposures. Another example would be persons living near hazardous waste sites. The distinction here is that some of the workers or some of the residents may be hypersusceptible, but all workers and all residents are at high risk because they have been exposed to levels higher than levels to which the general population is exposed.

In his book, Pollutants and High Risk Groups, Calabrese (1978) classifies hypersusceptible individuals into five main categories based on biological factors which increase human susceptibility to pollutants as described in Table 11. Mainly the first four categories will be considered at this time. Known physiological mechanisms form the basis of Calabrese's categorizations and include phenomena such as transplacental transfer of certain chemicals, greater gastrointestinal absorption in younger children, and vulnerability of the embryo and fetus. These factors clearly indicate that there is a wide range of susceptiblity in the human population.

Few data exist to aid in quantifying the amount of excess risk due to hypersusceptibility in humans. Such quantitation can usually be inferred from appropriate animal data, however, such data are scarce. Qualitative data, as well as the mechanistic processes, support the existence of such groups. Qualitative support is derived from observations in both animals and humans that adverse effects occur in only a portion of those exposed, despite similar exposures. The effects observed in the exposed population may also vary in degree of severity.

Despite the lack of quantitative dose-response information, hypersusceptible individuals have not been totally neglected in regulatory procedures.

TABLE 11

Biological Factors Predisposing Individuals to Hypersusceptibility to Pollutants*

Factors		Rationale
Developmental Processes		
Examples:	Pre- and Neonatal	Immature enzyme detoxification systems
	Young children	Higher rate of GI absorption
Genetic Disorders		
Examples:	G-6-PD deficiency	Hemolysis in presence of certain chemicals
	Sickle-cell trait	Predisposes toward hemolytic anemia
Nutritional Deficiencies		
Examples:	Vitamin C deficiency	Potentiates effects of pollutants
	Protein deficiency	Affects metabolism of insecticides
Existing Disease		
Examples:	Heart	Increased mortality in pollution episodes demonstrated in epidemiologic studies
	Lung	Existing conditions are aggravated by respiratory irritants
Behavioral Factors		
Examples:	Smoking	Increases exposures and therefore risk; interferes with normal cleansing mechanisms of lung
	Alcohol	Liver damage, synergism with other chemicals
	Dietary patterns	Increases exposures; deficiency states increase risk

*Source: Adapted from Calabrese, 1978

For example, the Clean Air Act of 1970 mandated that the health of hypersusceptible as well as healthy individuals of the population be protected. Two instances for which standards have been set for the hypersusceptible individual are nitrates and lead. For both contaminants, the hypersusceptibles are infants and children. Particularly in the case of nitrates, infants are the only group of the population affected. It appears that standards are set for the hypersusceptible group only if available human data demonstrate the risk to that particular group. If, however, animal models and/or mechanistic information suggest a hypersusceptible group, then this information can only be considered qualitatively in scientific guidance for risk assessment (e.g., the effect of PCBs or chlorinated hydrocarbons on pregnant women).

In general, the neglect of such subgroups is due to the lack of quantitative data and the assumption that those individuals comprise only a small proportion of the population. This latter issue will be discussed and assessed in detail in the following section of this presentation. However, although some of these groups may be quite small in number for a given chemical, the issue must be considered in light of potential exposure to complex mixtures. Since one or more specific hypersusceptible subgroups may be associated with each contaminant, the total number of individuals classified as hypersusceptible could comprise a significant proportion of the population. Thus, multiple chemical exposures could lead to multiple hypersusceptible subgroups. Furthermore, because of the diversity of each hypersusceptible subgroup, it has been postulated that everyone in the population will be a member of a susceptible group at certain times during life (Calabrese, 1978; Redmond, 1981).

Is the Present Approach Protective of Hypersusceptible Subgroups?

The EPA guidelines suggest that a 100-fold safety, or uncertainty, factor be incorporated when extrapolating from chronic animal studies to humans. This can be perceived as including a 10-fold factor to account for interspecies variability (i.e., extrapolation from animals to humans), and a 10-fold factor to account for intraspecies variability (i.e., the average vs. the sensitive human). To evaluate the extent to which the present approach protects hypersusceptible subgroups, the data source as well as the extrapolation process must be considered.

For the majority of chemicals, the sources of the data are laboratory experiments. Experimental animals are in-bred for physiological homogeneity. Further homogeneity is introduced by experimental designs that select animals of similar age and weight.

In an attempt to ascertain the extent to which a 10-fold dose-reduction protects sensitive members of the animal population, Dourson (1982) examined the range of log-dose probit slopes of 490 animal studies compiled by Weil (1972). The majority of the slopes, based on acute effects, were greater than 3. This would require a dose reduction of 10 to drop the average response at least three standard deviations, that is, to a level protective of the sensitive animal (Weil, 1972; Dourson, 1982). For those 8% of chemicals in the example having probit log-dose slope less than 3, a 10-fold reduction in dose would not achieve a three-standard-deviation reduction in response. Thus, the 10-fold factor may be protective of the sensitive laboratory animal, based on an assumed normal distribution of variablity for a majority of chemicals. There are few similar studies of the dose-response relationship in humans.

This 10-fold factor may not be adequate to protect humans because the human population is not as homogeneous as the animal population. Contributing to the heterogeneity in human populations is the fact that all members of the population are exposed to environmental pollutants regardless of health status, sex, age, weight or nutritional status. The range of individual variability of physiological values is ill-defined and is often not normally distributed in the healthy population. The variability for those who are diseased is generally much larger than that of the healthy population. The safety factor concept was developed in recognition of this variability as well as other uncertainties of the extrapolation procedure, but the selection of the number 10 is not based on a quantitative assessment of this variability.

The present approach has not been designed in ignorance of the existence of hypersusceptible individuals, but rather on the assumptions that 1) no quantitative data are available to support a more accurate dose reduction to protect them, and 2) that, for any one chemical, the portion of the population which is hypersusceptible is too small to merit consideration. The issues surrounding the former assumption merit further consideration and study. The issues surrounding the latter assumption are evaluated in the following section.

What Proportion of the Exposed Population is Hypersusceptible?

The hypothesis is that, for a population exposed to multiple chemicals, the proportion that can be considered hypersusceptible will be substantial. Various subgroups will be hypersusceptible to at least one of the chemicals and some to more than one. Therefore, while the present approach may be adequate for single chemicals, an alternative is necessary for the case of exposure to multiple chemicals.

There are two components to the process of quantitatively estimating the number hypersusceptible to toxic effects from exposure to multiple chemicals. The first step is the qualitative identification of those hypersusceptible subgroups. The second is to estimate the frequency of these groups.

Qualitative Identification of Hypersusceptible Groups.

The mechanistic approach of Calabrese (1978) is particularly useful for identifying potential hypersusceptible subgroups due to the absence of human and animal data to illustrate hypersusceptibility empirically for specific chemicals. Calabrese (1983) recognizes the scarcity of laboratory data and suggests potential animal models, several diseases, and conditions of proposed hypersusceptibility. Cardiovascular disease is the leading cause of death in the United States and a highly prevalent cause of morbidity. Although this disease has been widely studied, McCauley and Bull (1980) contend that the impact of environmental contamination on cardiovascular toxicity is not well known. Toxicological and epidemiological studies have shown an association between cardiovascular disease and environmental contaminants such as certain metal ions and chlorinated solvents. In some of the animal studies, healthy animals are used and exposure levels are quite high. On the other hand, human studies are unable to control for confounding factors such as diet and smoking habits. McCauley and Bull (1980) suggest animal models for various cardiovascular pathologies. Thus, animal studies offer the opportunity to control for the complexity of factors in the human environment and to define specific steps in the disease process which environmental toxins exacerbate. These authors further suggest animal models appropriate to the study of such environmental contamination.

The category of hypersusceptibility that has received the most attention in cases of environmental pollution is teratogenicity. The biological basis for embryonic and fetal vulnerability and for transplacental carcinogenicity is strong (Strobino et al., 1978; Rao et al., 1981, Saxena et al., 1981; Rice, 1981; Kurzel and Centrulo, 1981; Fabricant and Legator, 1981). However, the relationship between animal and human teratogenicity is not as clearly established as the relationship between animal and human carcinogenicity.

Through preliminary investigations we have identified several chemicals to support the hypothesis that several subgroups are hypersusceptible to environmental exposures. Table 12 shows these chemicals. In addition to the embryo, fetus and young children, those with existing pulmonary disease and coronary heart disease are likely candidates for high prevalence subgroups.

Estimating Prevalence of Hypersusceptible Subgroups.

While statistics on births and mortality are routinely collected, statistics on morbidity are not. The prevalence of chronic conditions is available from epidemiologic studies and national surveys (e.g., Framingham, HANES, HIS) which are not collected by political division as are vital statistics. Furthermore, the epidemiologic surveys are irregularly performed on a national sample, whereas to vital statistics are compiled every year for the entire population.

Table 12 shows the prevalence of several specific hypersusceptible subgroups. Morbidity rates from an interview survey conducted by the National Center for Health Statistics (1974) were: chronic bronchitis -- 33 per 1000; hypertensive disease -- 60 per 1000; and heart conditions -- 50 per 1000. This interview did not include residential institutions such as

TABLE 12

Prevalence of Subgroups Hypersusceptible to Effects of Common Pollutants

Hypersusceptible	Prevalence[a]	Chemicals[b]	Reference[b]
Embryo, fetus, neonate	pregnant women: 21/1000[c]	Carcinogens, solvents, CO, mercury, lead, PCBs, pesticides	Rice, 1981; Kurzel and Cetrulo, 1981; Saxena et al., 1981
Young children	ages 1-4: 70/1000	Hepatotoxins, PCBs, metals	Calabrese, 1981; Friberg et al., 1979
Lung disease	emphysema, asthma: 37/1000[d]	Ozone, Cd, particulates, SO_2, NO_2	Holland, 1979; Redmond, 1981
Coronary heart disease	coronary heart disease: 16-27/1000[d]	Chlorinated solvents, fluorocarbons	McCauley and Bull, 1980; Aviado, 1978
Liver disease	liver condition: 20/1000[e]	Carbon tetrachloride, PCBs, insecticides, carcinogens	Calabrese, 1978

---

[a] All estimates based on 1970 census

[b] Representative sample. Some evidence from animal studies only.

[c] Authors' estimate from 1970 census statistics data

[d] Health Interview Survey (NCHS, 1970)

[e] Health Interview Survey (NCHS, 1975)

nursing homes and penitentiaries, and is believed to underestimate the true prevalence in the general population. Therefore, the potential number at risk may be higher.

The proportion of hypersusceptible individuals in an exposed population may be estimated by multiplying the exposed population by the proportion at risk in the general study population. Although greater precision could be obtained by partitioning the exposed population into demographic subgroups at differing risks and calculating age-, race- and sex-specific risks, this is generally believed to be impractical. Thus, estimates of the prevalence of hypersusceptible subgroups for a given site will be imprecise. For this reason a risk assessment scheme for cases of exposure to multiple chemicals may be limited to an ordinal or ranking scheme rather than one which is based on continuous data.

When considering exposure to more than one chemical, the number of hypersusceptible subgroups is even greater. Table 13 shows inventories of typical waste sites along with the prevalence of the high-risk subgroups. One method of estimating the proportion hypersusceptible to more than one chemical is to add the rates for each chemical. The proportion at high risk is conservatively estimated to be from 10-20% among these sites. The number of higher risk individuals for a hypothetical population of 5000 is given in column 4. Clearly, some of the categories of chronic diseases are not mutually exclusive, and addition would provide an overestimate since some individuals can be the victims of more than one condition. On the other hand, certain individuals may be excessively hypersusceptible to the effects of one chemical due to more than one pre-existing condition.

TABLE 13

Hypersusceptible Subgroups Associated with Typical Inventory of Chemicals at Waste Sites to Which People Have Been Exposed

Occurrence	Chemical	Hypersusceptible Subgroup	Prevalence Rate (per 1000)	Number of Hypersusceptibles in Hypothetical Population of 5000
4 of 4 sites	Chlorinated ethanes (dichloroethanes)	CHD	CHD: 24	120
		liver condition	liver condition: 20	100
		pre-exposure to hepatotoxins		
		embryo/fetus	pregnant women: 21	105
	Dichloroethylenes	CHD		
	1,1,1-Trichloroethane	CHD		
		liver condition		
		pre-exposure to hepatotoxins		
	Trichloroethylene	liver condition		
	Chloroform	CHD		
		lung	lung (bronchitis): 33	165
3 of 4 sites	Benzene	thalassemia plus others	thalassemia: 0.1-8% of persons of Italian, Greek, Syrian and African origin	
	Ethyl benzene	thalassemia plus others		
	Methylene chloride	CHD	CHD: 24	120
		liver condition	liver condition: 20	100
	Tetrachloroethylene	lung	lung (bronchitis): 33	165
		liver condition		

TABLE 13 (cont.)

Occurrence	Chemical	Hypersusceptible Subgroup	Prevalence Rate (per 1000)	Number of Hypersusceptibles in Hypothetical Population of 5000
2 of 4 sites	Carbon tetrachloride	liver condition plus others	liver condition: 20	100
	Trichlorofluoromethane	lung	lung (bronchitis): 33	165
	Vinyl chloride	CHD	CHD: 24	120
	Arsenic	vitamin C deficiency	vitamin C deficiency: 10-30% of infants, children and adults of low income	25
	Chromium	young children (to metals)	ages 1-4: 70	350
	Selenium	vitamin E deficiency		
	Silver	selenium deficiency		
	Mercury	vitamin C deficiency cystinuria, pregnant women plus others		105

Conclusion

Due to the large number of individuals in high risk subgroups the present approach may be adequate only for single chemicals. An alternative is necessary when evaluating the health risk from exposure to multiple chemicals. Some of the hypersusceptible subgroups for single chemicals may comprise a significant proportion of the population while others may not. However, when considering multiple chemical exposures and, therefore, multiple hypersusceptible subgroups, even the very small groups become significant because they are part of a large number of individuals who are hypersusceptible to the chemical mixture. For a typical waste site, 10-20% of the population may be at high risk.

Because the importance of considering such groups is evident, a scheme has been devised to systematically assess the vulnerability of high-risk groups to exposure to multiple chemicals for a defined site. The scheme is proposed to derive an index for a hazardous waste site on an ordinal scale (Table 14). The proposed approach involves identification of the contaminants and their respective hypersusceptible subgroups. Once these subgroups have been identified, the proportion of the population they comprise will be estimated by applying prevalence rates for the general population to the exposed population. Methods of merging the affected population with the exposure data will be explored. This overall ranking scheme is envisioned as a component of the multiple chemical risk assessment procedure.

TABLE 14

Proposed Approach to Evaluate Multiple Chemical Exposures for Impact on Hypersusceptible Subgroups

---

1) Obtain monitoring data relative to the exposure

2) List the hypersusceptible subgroups associated with each contaminant

3) List the prevalence rates for each hypersusceptible subgroup

4) Calculate (from 2 and 3), the estimated percentage and number of hypersusceptible individuals in the exposed population

5) Incorporate this index into the site-specific risk assessment

---

CRITIQUES

DR. JAMES WITHEY

The following are philosophical approaches to dealing with subpopulations at greater risk:

1. Ban exposure to substance. Tell people they can't live near dump site.
2. Clean up site. (This may not be cost-effective.)
3. Recommend that smokers and drinkers be looked at specifically.
4. Some groups that are particularly sensitive may be discovered if good epidemiological programs and followup are in place.
5. Consider those people who are exposed to chemicals with long elimination half-life, like DDT. Such chemicals may accumulate in large amounts in storage depots of exposed individuals and then become mobilized during sickness or weight loss.

DR. EULA BINGHAM

In her presentation, Dr. Erdreich presented the following definition of hypersusceptible:

> A hypersusceptible individual (or populations) is one who will experience an adverse health effect to one or more pollutants, significantly before the general population, because of one or more factors which predispose the individual to the harmful effects.

I agree with this definition -- actually we are asking whether certain groups are at "higher risk." These groups have been identified by various methods and may be characterized as follows:

1. Developmental -- (i.e., various periods in life cycle) -- e.g., conceptus, children, reproductively active males and females, aging populations.
2. Dietary influences -- nutritional aspects.
3. Disease states -- e.g., impaired renal function, asthmatics, chronic pulmonary disease, hypertension, etc.

4. Previous exposures (usually occupational) -- e.g., lead burden, initiating doses of carcinogens.

5. Genetic --

   AHH induction vs. human lung cancer

   Thalassemias -- deficiency in production of RBCs, homozygons vs. heterozygons

   25% Italian-American and German-American

   Sickle cell trait --

   G-6-PD deficiency -- frequency may be 15%

All individuals will be more susceptible at one time or another. I agree with this statement, but I don't agree that as a hypersusceptible group, the conceptus has received most attention. Societal responses and the high media value make it a visible area, but actually from a scientific viewpoint, skin sensitization, enzyme disfunctions and allergic reactions, other than skin, have received great attention.

A particular note of caution is that when you use a TLV in any way to set acceptable levels, it should be remembered that not only are they based on a limited period of exposure, but are usually set for young, healthy, adult, white males.

The next questions are:

1. What are the sizes of the subgroups and what constitutes a "significant" numbers of hypersusceptible individuals?
2. How much more susceptible are they, e.g., 10 times, 100 times, 1000 times? (Risk is 16 and 36 times greater in medium and high inducers of AHH.)

In the present approach by the Agency:

1. Quantitative data to support the thesis have not been presented and cannot be found in the literature.

2. Any one chemical may be quite small in the number of susceptibles, but would merit consideration in combinations (as in dump sites).

A review of the literature to determine the magnitude of the increased risks incurred by "susceptible" groups will provide some insight into the appropriate safety factor. It seems likely that for some contaminants the so-called susceptible groups will be so numerous if a lifecycle viewpoint is used, that the "nonsusceptible" or more resistant becomes the minority of a population.

DR. MAGNUS PISCATOR

The following are some parameters of interest when a population is exposed to a contaminant in water:

1. The contaminant may occur in one water supply used by the whole community. The concentrations in tap water will be similar in all households. If each household has its own well, the distribution of concentrations of the contaminant will probably be log-normal; and a few wells may have quite high concentrations. If several contaminants are present, they may move with different speeds into the water supply, and peak concentrations may thus occur at different times.
2. The ingested dose will be dependent on water consumption, which may vary between 0.5 and 3 ℓ/day. The distribution is probably normal.
3. The absorbed dose will depend on age, diet, existence of nutritional deficiencies and diseases, and probably many other factors. Some substances may be expected to always be absorbed to more than 90%, whereas others may show a very large variation in absorption, e.g., lead may be expected to show a log-normal absorption distribution, with infants and women with iron and calcium deficiencies at the extreme end of the distribution. An additional dose may be obtained by inhalation, if the substance easily evaporates from water.

4. The biological half-time may vary due to age, existence of liver or kidney disease, interactions, diet, etc. Some compounds are more rapidly metabolized by children, people already exposed to some chemicals, e.g., drugs.. Both normal, log-normal and bimodal distributions may exist.
5. The critical concentration for effects on an organ may vary considerably. This may be due to genetic, nutritional and disease factors. The cardiomyopathy seen in beer drinkers exposed to cobalt may serve as an example. Asthmatics may get lung irritation from concentrations of air pollutants tolerated by healthy people.
6. The final result is a wide variation in effects, the distribution being log-normal, with a majority showing no or mild effects, whereas a few may be expected to get some more marked effects. Most populations exposed to waste chemicals are small and it is unlikely that in such small groups any significant adverse effects will be noted.
7. The decisive factor is obviously the ingested dose. If it is below a certain level, none of the other factors will have enough influence to create an effect.

DISCUSSION

DR. JULIAN ANDELMAN

In the presentation on assessment of exposure by Dr. Nisbet (see previous chapter), as well as the critique by Dr. Piscator on subpopulations at greater risk, the likelihood of encountering log-normal exposures and uptakes in an exposed population was appropriately raised.

Thus, in addition to considering the increased sensitivity of certain segments of the exposed population, particular attention should be directed to the question of the variability of exposures and body burdens among groups that might nominally be expected to have the same exposure. Such variabilities can and do arise as a result of individual behavior, as well as varied physiology. This is graphically shown in geographically discrete populations of children who have log-normally distributed concentrations of lead in their blood. An example of the implication of this phenomenon is the assessment of the likely exceedance of a threshold level (e.g., NOAEL) via a given route of exposure, such as drinking water. Assuming the water concentration and a 2 ℓ water intake would correspond to this NOAEL, the fraction of the population likely to exhibit an effect might be considerably less than 100% due to the log-normal question. This could be addressed, however, through the uncertainty factor mechanism, but should be specifically considered as a principle to be incorporated in some fashion.

DR. MARVIN LEGATOR

The area of the susceptible individual as discussed at this meeting presented an interesting approach to linking individual chemicals to a possible adverse effect on subjects in the population. It may well be that establishing such linkages could offer a procedure for detecting an adverse outcome before it is more generally apparent.

DR. REVA RUBENSTEIN

The paper on hypersusceptible individuals was very thought-provoking. It seems to me that examination of clinical case studies of individuals with underlying pathology may be a fruitful way of addressing both "hypersusceptibility" and multichemical exposure. Many of these patients are already receiving multiple drug therapy. An additional, possibly productive line of investigation is to better characterize, or classify, the known drug interactions in "normal" populations.

DR. MAGNUS PISCATOR

The main emphasis of the presentation was on preexisting disease, and certain groups were identified. In my critique I pointed out that the final effect will be dependent on a number of exogenous and endogenous factors, all of which will be within normal or log-normal distributions, e.g., concentration of contaminant in water, intake of contaminant, absorption, biological half-time, critical concentration.

I also showed that in a subgroup with cardiovascular disease treated with a phenoxypropionic acid, additional exposure to a small amount of phenoxy acids would have little significance, whereas the exposure might be significant in a healthy population.

People with preexisting disease generally are taking drugs, and interference with drug metabolism may occur, changing elimination. Thus exposure to PAH, DDT, etc., may decrease half-times for some drugs and make treatment less effective.

DR. JAMES WITHEY

Some of the newer topics such as the "Special Groups at Risk" need to be refined a little and the approach crystalized. Undoubtedly, the "dumps" will have to be considered on a case-by-case basis, and "special groups" may be identified as a consequence of the retrospective epidemiology.

DR. RICHARD KOCIBA

The issue of hypersusceptible subpopulations should consider these subpopulations as the extreme ends of the normal distribution curve of the population as a whole. On this basis, there appears, in most cases, to be sufficient adequacy in the conventional uncertainty factors used to historically accomodate intraspecies variability.

One must keep in mind that the entire population (and the resultant normal distribution curves) can be considered to be comprised of various subgroups of varying susceptibility. Based on these factors, there does not appear to be any great need to change the basic approach that has been used historically (uncertainty factors) to accommodate intraspecies variability.

In regard to the issue of multichemical carcinogenesis, the unpublished studies discussed by Dr. Schneiderman in his critique of the presentation on biological bases of toxicant interactions should be reviewed. This would allow one to evaluate the likely outcome of multichemical long-term exposure with regard to carcinogenesis.

DR. IAN NISBET

Hypersusceptible groups are the only groups of importance for risk assessment because they constitute the low end of the dose-response curve, by definition. If a hypersusceptible subpopulation is sufficiently discrete that the distribution of susceptibilities is bimodal [Figure 26(A)], then the dose-response curve may be convex upwards. If so, the assumption that a linear dose-response relationship is an upper bound on risk will be wrong [see Figure 26(B)].

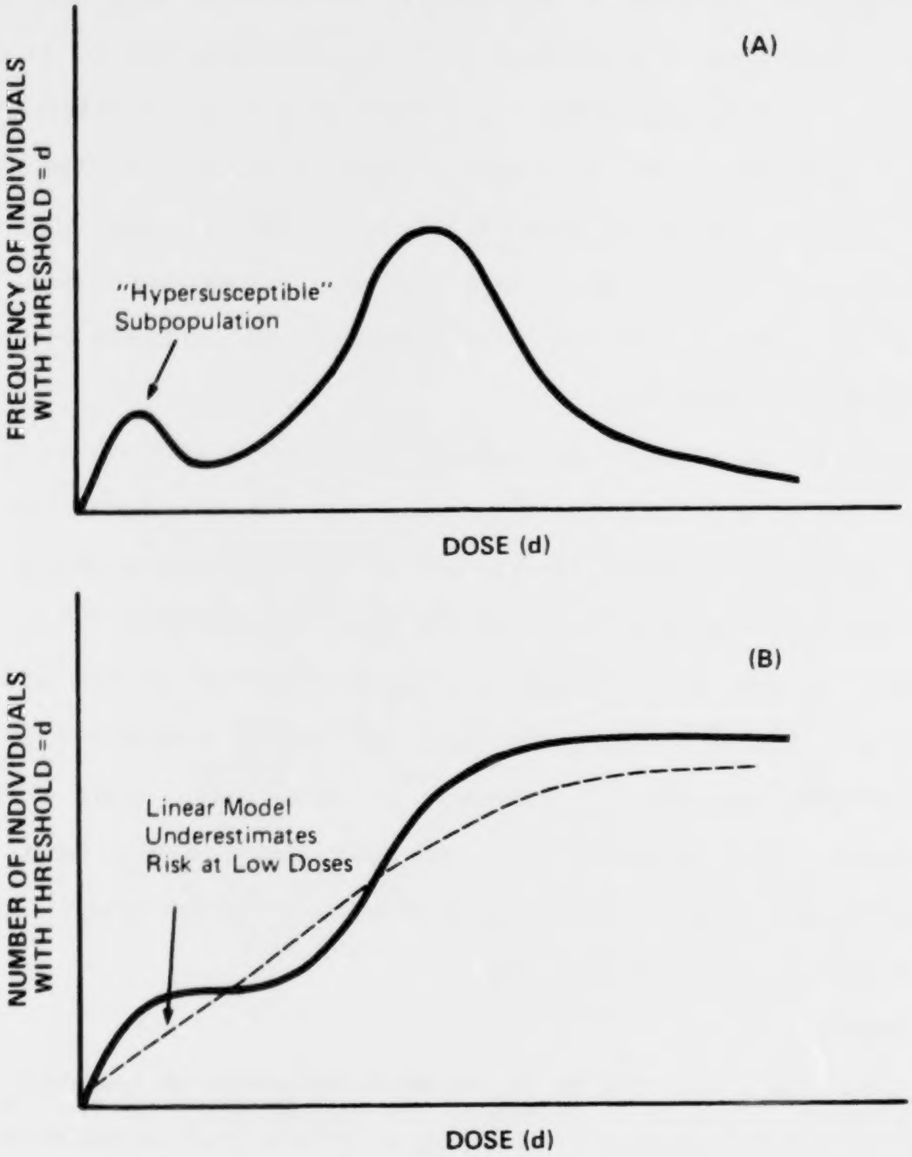


FIGURE 26

Dose-response characteristics of a hypothetical population that includes a hypersusceptible subpopulation. (A) Frequency of individuals susceptible to dose d (i.e., for whom d is a threshold). (B) Comparison of linear dose-response model with dose-response curve for hypothetical population.

DR. MARVIN SCHNEIDERMAN

With respect to the issue of sensitivity, two pieces of data that I had difficulty interpreting or understanding in the past now look as if they might be interpreted to show either the effects of greater sensitivity in a portion of a human population, or greater responsiveness in a portion of the population perhaps induced by other exposures. That is, these data may provide evidence of an effect of mixed exposures in a human population. The two sets of data derive from industrial exposures. The attached Figure 27 shows these data, schematically.

Both parts of the figure show responses (or relative risk) in an industrial population as a function of duration of exposure. In each of the two parts the points representing the persons with the lowest exposures lie above the dose-response line fitted to the data. One possible interpretation of this is that these "excessive responses" include persons who had been previously exposed to other materials, thus making them more sensitive to the subsequent exposure (of asbestos, or radon gas). There are, of course, several other "explanations": the sick worker effect (what made them "sick"?); poor data for the "controls" or zero-exposed groups; underestimate of dose for low exposures, etc.

GENERAL COMMENTS

- The intent should not only be to estimate the number of individuals in each subgroup but also to determine the health impact on the overall population. Both frequency and severity of effects must be addressed.
- Susceptibility can be differences in kind as well as degree.
- Susceptibility may be bimodal, which would put into question the slope of the usual dose-response curve.
- Multiple causation must be considered. People with preexisting health problems may be the first ones affected. An additional insult (e.g., chemical exposure) may be overlooked and their disease will be blamed on the preexisting condition (e.g., alcoholism, problems associated with smoking).

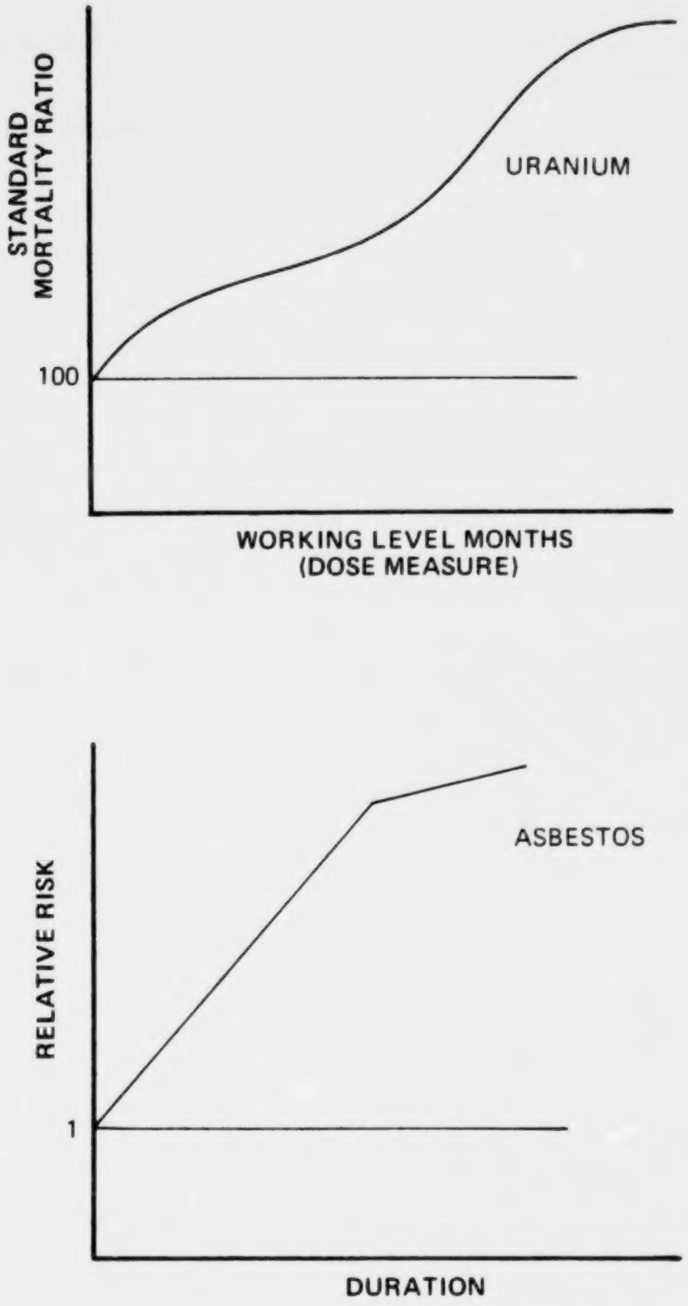


FIGURE 27

Response (or risk) in an Industrial Population as a Function of Duration of Exposure to Uranium and Asbestos

Source: Adapted from Lundin et al., 1971 and Nicholson et al., 1982

- Subgroups should be identified and used to indicate adverse effects only when it is appropriate.

- Adding all hypersusceptible populations together should not be considered.

- The most sensitive animal model could be used.

REFERENCES

Aviado, D.M. 1978. Effects of fluorocarbons, chlorinated solvents, and inosine on the cardiopulmonary system. Environ. Health Perspect. 26: 207-215.

Calabrese, E.J. 1978. Pollutants and High-Risk Groups. The Biological Basis of Increased Human Susceptibility to Environmental and Occupational Pollutants. John Wiley and Sons, NY.

Calabrese, E.J. 1981. Nutrition and Environmental Health: The Influence of Nutritional Status on Pollutant Toxicity and Carcinogenicity. John Wiley and Sons, NY.

Calabrese, E.J. 1983. Principles of Animal Extrapolation. John Wiley and Sons, NY.

Dourson, M.L. 1982. Regulatory and experimental support of safety factors. (Submitted for publication)

Fabricant, J.D. and M.S. Legator. 1981. Etiology, role and detection of chromosomal abberations in man. J. Occup. Med. 23: 617-625.

Friberg, et al., Ed. 1979. Handbook on the Toxicology of Metals. Elsevier/North Holland Biomedical Press.

Holland, W.W., A.E. Bennett, I.R. Cameron, et al. 1979. Health effects of particulate pollutants: Reappraising the evidence. Am. J. Epidemiol. 110: 525-659.

Kline, J.K., Z.A. Stein, B.R. Strobino, M.W. Sussex and D. Warburton. 1977. Surveillance of spontaneous abortions: Power in environmental monitoring. Am. J. Epidemiol. 106: 345-350.

Kurzel, R.B. and C.L. Cetrulo. 1981. The effect of environmental pollutants on human reproduction, including birth defects. Environ. Sci. Technol. 15: 626-640.

Lundin, F.E., J.K. Wagoner and V.E. Archer. 1971. Radon daughter exposure and respiratory cancer. Quantitative and temporal aspects. NIOSH/NIEHS Joint Monograph No. 1. NTIS, Springfield, VA.

Maclure, K.M. and B. MacMahon. 1980. An epidemiologic perspective of environmental carcinogenesis. Epidemiol. Rev. 2: 49-70.

Mantel, N. and M. Schneidermann. 1975. Estimating safe levels, a hazardous undertaking. Cancer Res. 35: 1379-1386.

McCauley, P.T. and R.J. Bull. 1980. Experimental approaches to evaluating the role of environmental factors in the development of cardiovascular disease. J. Environ. Pathol. Toxicol. 4: 27-50.

NCHS (National Center for Health Statistics). 1970. Natality Statistics Analysis, United States, 1965-1967. Vital and Health Statistics. PHS Publ. No. 1000, Series 21 - No. 19. NCHS, PHS, Washington, U.S. GPO, May.

NCHS (National Center for Health Statistics). 1974. Prevalence of Chronic Circulatory Conditions, United States, 1972. Vital and Health Statistics Series 10 - No. 93. NCHS, PHS, Washington, U.S. GPO, September.

NCHS (National Center for Health Statistics). 1975. Selected Vital and Health Statistics in Poverty and Nonpoverty Areas of 19 Large Cities, United States, 1969-1971. Vital and Health Statistics Series 21 - No. 26. NCHS, PHS, Washington, U.S. GPO, November.

Nicholson, W.J., G. Perkel and I.J. Selikoff. 1982. Occupational mortality to asbestos: Population at risk and projected mortality 1980-2030. Am. J. Ind. Med. 3: 259-311.

Ohio Department of Health Report of Vital Statistics for Ohio, 1975. Columbus, OH.

Rao, K.S., B.A. Schievetz and C.N. Park. 1981. Reproductive toxicity risk assessment of chemicals. Vet. Human Toxicol. 23: 167-175.

Redmond, C.K. 1981. Sensitive population subsets in relation to effects of low doses. Environ. Health Perspect. 42: 137-140.

Rice, J.M. 1981. Prenatal susceptibility to carcinogenesis by xenobiotic substances including vinyl chloride. Environ. Health Perspect. 41: 179-188.

Saxena, M.C., J.K.J. Siddiqui, A.K. Bhargaua, C.R. Krishna Murti and D. Kutty. 1981. Placental transfer of pesticides in humans. Arch. Toxicol. 48: 127-134.

Strobino, B.R., J. Kline and Z. Stein. 1978. Chemical and physical exposures of parents: Effects on human reproduction and offspring. Early Human Development. 1: 371-399.

Weil, C.S. 1972. Statistics vs. safety factors and scientific judgement in the evaluation of safety for man. Toxicol. Appl. Pharmacol. 21: 454-463.

HEALTH ASSESSMENT OF EXPOSURES TO CHEMICAL MIXTURES

Biological Bases of Toxicant Interactions and Mathematic Models

Presentation:	Dr. Patrick Durkin Syracuse Research Corporation
Critique:	Dr. Thomas Clarkson University of Rochester
Critique:	Dr. Herbert Cornish University of Michigan
Critique:	Dr. Kenneth Crump Science Research Systems
Critique:	Dr. Marvin Schneiderman Clement Associates

PRESENTATION

DR. PATRICK DURKIN: MULTIPLE CHEMICAL EXPOSURES

Introduction

Having addressed the issues of single chemical risk assessments from multiple routes of exposure, the next and last step is to determine a reasonable approach or set of approaches for dealing with multiple chemical exposures. While some hazardous waste disposal facilities may involve significant exposure to only a single chemical, most hazardous waste disposal facilities will involve exposures to a variety of compounds that may induce similar or dissimilar effects. For the purposes of this discussion, it will be assumed that the compounds at the site have been identified, single compound risk assessments have been conducted as described in the previous chapters, exposure levels for the population at risk have been determined, and the available data on toxicant interactions have been analyzed. This section will discuss the biological and chemical bases for assuming that toxicant interactions may occur, describe mathematic models which can be used to assess the effects of multiple compound exposure, give examples and indices for quantifying toxicant interactions, and recommend an approach for hazardous waste disposal facilities.

Biological and Chemical Bases of Toxicant Interactions

The ability to predict how specific mixtures of toxicants will interact must be based on an understanding of the mechanisms of such interactions. Most reviews and texts which discuss toxicant interactions make some attempt to discuss the biological or chemical bases of the interactions (e.g., Klaassen and Doull, 1980; Levine, 1973; Goldstein et al., 1974; NRC, 1980; Veldstra, 1956; Withey, 1981). Although different authors use somewhat different classification schemes for discussing the ways in which toxicants interact, it is generally recognized that toxicant interactions may be based on any of the processes that are significant to the toxicologic expression of a single compound: absorption, distribution, metabolism, excretion, and activity at the receptor site(s). In addition, compounds may interact chemically, causing a change in the biological effect, or they may interact by causing different effects at different receptor sites. Using a modification of the basic scheme proposed by Veldstra (1956), Table 15 summarizes these general modes of interaction along with some examples. As indicated in the discussion below, there is some overlap among the different categories.

Most cases of direct chemical-chemical interactions lead to a decrease in toxicologic activity, and this is one of the common principles of antidotal treatment. Examples include the use of chelating agents to complex with metal ions, the inactivation of heparin by protamine, and the use of ammonia as an antidote to the ingestion of formaldehyde through the formation of hexamethylenetetramine (Goldstein et al., 1974). This class of reactions has been referred to as chemical antagonism by Klaassen and Doull (1980). Chemical reactions which lead to greater than additive effects appear to be less common and are certainly much less documented. One example which has recently received considerable attention is the formation of nitrosamines from nitrites and amines, which results in an increase in both toxic and carcinogenic effects (Weisburger and Williams, 1980). Thus, while antagonism may be predominant in this type of toxicant interaction, synergism or potentiation cannot be ruled out.

Many examples of toxicant interactions are based on alterations in patterns of adsorption, distribution, excretion, or metabolism of one or more compounds in the mixture. A recent review of these factors in the assessment of multiple chemical exposures has been presented by Withey (1981).

TABLE 15

Chemical and Biological Bases of Toxicant Interactions*

	Examples	
Bases of Interaction	Synergism or Potentiation	Antagonism
Chemical	Formation of nitrosamines from nitrites and amines	Chelating agents and metals
Biological		
Absorption	Increased dermal absorption of many toxicants when administered in dimethyl sulfoxide	Decreased absorption of tetracycline when administered with calcium carbonate
Distribution	Displacement of anticoagulants from plasma proteins by phenylbutazone	
Excretion	Decreased renal excretion of penicillin when co-administered with probenecid	Increased renal elimination of phenobarbital when co-administered with sodium bicarbonate
Metabolism	Increased toxicity of parathion by simulation of microsomal enzyme activity with phenobarbital	Decreased toxicity of parathion by inhibition of microsomal enzyme activity with piperonyl butoxide
Interaction at Receptor Sites (Receptor Antagonism)		Blocking of acetylcholine receptor sites by atropine after poisoning with organophosphates
Interaction Among Receptor Sites (Functional Antagonism)		Interaction of histamine and norepinephrine on vasodilation and blood pressure

*See text for discussion, additional examples, and references.

All of these types of interactions essentially alter the bioavailability of the toxic agent(s) at the receptor site(s) without qualitatively affecting the toxicant-receptor site interaction.

Most types of interactions based on alterations in absorption involve vehicle effects, the chemical formation of poorly absorbed conjugates, or decreases in gastrointestinal motility. For instance, dimethyl sulfoxide, a commonly used vehicle in dermal toxicity studies, is known to facilitate the absorption of many organic compounds across the skin, thus causing apparent potentiation (Goldstein et al., 1974). Similarly, the acute oral toxicity of many compounds is substantially affected by the vehicle used, and a large number of these effects are probably due to differences in rate of absorption. Examples of compounds that form poorly absorbed complexes after oral administration include tetracycline and calcium carbonate, as well as cholestyramine and cholesterol (Goldstein et al., 1974). Some compounds, such as codeine, morphine, atropine, and chloroquine decrease the rate of gastric emptying and thus decrease the rate of absorption of orally administered compounds. For the most part, such interactions usually lead to decreases in effects due to the slower rate of absorption, rather than increases in effects due to more complete absorption (Levine, 1973). As discussed by Withey (1981), there are relatively few examples of toxicologically significant changes in absorption associated with the inhalation of mixtures.

Distribution can play a role in compound interactions if a more active agent is displaced from an inactive site to a primary receptor site by a less active or inactive agent. One of the best documented examples of this type of activity is the displacement of anticoagulants from plasma proteins by compounds such as barbiturates, analgesics, antibiotics, or diuretics

(Goldstein et al., 1974). Since body fat represents a major inactive storage site for many lipophilic xenobiotics, it may be anticipated that compounds which cause fat mobilization could result in similar potentiating effects (Withey, 1981). It should be noted that both of the above types of examples result in greater than additive effects -- synergism or potentiation. A distributional mechanism for antagonism does not seem probable and has not been encountered in the literature reviewed.

Excretion as a basis for toxicant interaction usually involves compounds which are eliminated via the kidneys. For instance, probenecid or carinamide both competitively inhibit the elimination of penicillin, thus prolonging or potentiating its desirable therapeutic effect. Similarly, phenylbutazone inhibits the renal excretion of hydroxyhexamide, which can cause undesirably prolonged hypoglycemia (Goldstein et al., 1974). If a toxicant is eliminated via the kidneys, a stimulation of renal elimination can cause an antagonistic effect, as is seen with the coadministration of phenobarbital and sodium bicarbonate in which the increased urine alkalinity induced by the bicarbonate ion increases the excretion of phenobarbital.

Altered patterns of compound metabolism have been shown to be the bases of many toxicant interactions. The major enzyme system involved in such interactions is liver microsomal mixed-function oxidase which is involved in the activation or detoxication of a wide variety of compounds and can be induced by agents such as phenobarbital and inhibited by agents such as piperonyl butoxide (Goldstein et al., 1974). Thus, depending on whether or not the toxicant is activated or detoxified, inducers or inhibitors of this enzyme system can cause synergistic/potentiating effects or antagonistic effects. However, toxicant interactions involving this enzyme system can be very complex and are dependent on both dose and duration of exposure, with some compounds causing an initial inhibition of enzyme activity followed by a marked induction of activity (NRC, 1980). Although liver microsomal mixed-function oxidase is the most commonly studied enzyme system involved in toxicant interactions, mixed-function oxidases in other tissues may also play an important role in toxicant interactions, as may other enzyme systems such as alcohol and aldehyde dehydrogenases, monamine and diamine oxidases, dehydrochlorinases, azo and nitro reductases, hydrolases as well as enzyme systems involved in conjugation reactions. For instance, ethanol is a useful antagonist for the toxic effects of methanol by competitive substrate inhibition of alcohol dehydrogenase, suppressing the formation of formaldehyde and formic acid from methanol (Goldstein et al., 1974).

As indicated above, all of these biological modes of toxicant interactions -- absorption, distribution, excretion, and metabolism -- are essentially dispositional, affecting the amount(s) of toxicant(s) reaching the primary receptor(s), and most of these types of interactions can involve either synergism/potentiation or antagonism. The other basic type of biological bases for toxicant interactions involves events that occur at the receptor sites or among the receptor sites, and are usually thought to result solely in antagonistic interactions. The antagonistic nature of interactions that occur at the same receptor site has been discussed by Veldstra (1956):

> ...we may say that the effect of a combined action of two compounds at the same site of primary action will not result in a synergism, but will, generally, even be unfavorable. The competition for the receptor will usually decrease the frequency of the best interactions, and with decreasing intrinsic activity of one of the components the combined action will more and more take the form of a competitive antagonism.

Examples of such interaction include the antagonistic effects of oxygen on carbon monoxide, atropine on cholinesterase inhibitors, and naloxone on morphine (Goldstein et al., 1974). The antagonistic consequences of this type of toxicant interaction are so consistent that it has been termed receptor antagonism by Klaassen and Doull (1980) and pharmacologic antagonism by Levine (1973). While it does not seem inconceivable that one compound could increase the intrinsic activity of another compound by modifying the receptor site -- analogous to the effect of modulators on regulatory enzymes -- such interactions have not been demonstrated.

Interaction among receptor sites is also thought to result primarily in antagonistic effects and has been referred to as functional antagonism by both Klaassen and Doull (1980) and Levine (1973). This type of interaction is most commonly defined as two or more compounds acting on different receptor sites and causing opposite effects on the same physiologic function. Examples include the opposite effects of histidine and norepinephrine on vasodilation and blood pressure, and the anticonvulsive effects of barbiturates on many compounds that cause convulsions. However, that interactions among receptor sites uniformly result in an antagonistic response is not certain, particularly when the receptor sites act on different physiological systems. The rationale for this statement has been presented by Veldstra (1956):

> The sites of action for two compounds having the same type of activity may be different. This is the case when the effect can be caused either by a direct stimulation or by the annihilation of an inhibition.
>
> Competitive antagonists for different intermediates in a biosynthetic chain fall in the same category, if the inhibition of the synthesis of the end-product is taken as their effect.
>
> In both cases, the combination of two compounds, linked in parallel or in series, as it were, may well result in a synergistic effect.

> When the components of a combination possess different sites of action and different types of activity, no plausible prediction about the possibility of synergism can be made, unless their mode of action is well known.

A possible illustration of Veldstra's argument is presented in the work of Alstott et al. (1973), who examined the acute lethal effects of combinations of 1-methylxanthine and ethanol on mice, and noted two basic types of effects: kidney dysfunction and increased respiratory rate and depth. In organisms exposed to mixtures in which the ratio of 1-methylxanthine to ethanol was relatively high, antagonism of acute lethal toxicity was observed. However, in mixtures in which the same ratio was relatively low, a synergism of acute lethal toxicity was observed. This indicates that in cases where toxicants interact at more than one receptor site, the nature of the interaction can be either antagonistic or synergistic. The complicating factor of the "asymmetric" pattern of interaction observed by Alstott et al. (1973) is discussed in greater detail in the following section.

Mathematic Models for Joint Action

The simplest mathematic models for joint action describe either dose addition or response addition. Dose addition, referred to as simple similar action by Finney (1971) and simple joint action by Bliss (1939), assumes that the toxicants in a mixture behave as if they were dilutions or concentrations of each other, thus the slopes of the dose-response curves for the individual compounds are identical and the response elicited by the mixture can be predicted by summing the individual doses after adjusting for differences in potency, the ratio of equitoxic doses. Although this assumption can be applied to any model (e.g., the one-hit model in NRC, 1980), it has been most often used in toxicology with the log-dose probit-response model which will be used to illustrate the assumption of dose additivity. Assume that two toxicants show the following log-dose probit-response equations:

$$Y_1 = 0.3 + 3 \log Z_1 \quad (1)$$

$$Y_2 = 1.2 + 3 \log Z_2 \quad (2)$$

where Y_i is the probit response associated with a dose of Z_i. The potency, p, of toxicant-2 with respect to toxicant-1 is, by definition, Z_1/Z_2 when $Y_1 = Y_2$ (i.e., equitoxic doses). In this example, the potency, p, is ~2. Dose addition assumes that the response, Y, to any mixture of the two toxicants can be predicted by:

$$Y = 0.3 + 3 \log (Z_1 + pZ_2) \quad (3)$$

It should be noted that since p is defined as Z_1/Z_2, Equation 3 essentially converts Z_2 into an equivalent dose of Z_1 by adjusting for the difference in potency. A more generalized form of this equation for any number of toxicants is:

$$Y = a_1 + b \log (\Sigma f_i p_i) + b \log Z \quad (4)$$

where a_1 is the y-intercept of the dose-response equation for toxicant-1, b is the slope of the dose-response line for each toxicant, f_i is the proportion of the i^{th} toxicant in the mixture, p_i is the potency of the i^{th}-toxicant with respect to toxicant-i (Z_1/Z_i), and Z is the sum of the individual doses in the mixture. A more detailed discussion of the derivation of the equations for dose addition is presented by Finney (1971).

The other form of additivity is referred to as response addition. As detailed by Bliss (1939), this type of joint action assumes that the two toxicants act on different receptor systems and that the correlation of individual tolerances may range from completely negative (r = -1) to completely positive (r = +1) correlation. Analogous to the concept of dose addition, response addition assumes that the response to a given concentration of a mixture of toxicants is completely determined by the responses to the components and the correlation coefficient. Taking P_3 as the proportion of organisms responding to a mixture of two toxicants which evoke individual responses of P_1 and P_2

$$P_3 = P_1 \text{ if } r = 1 \text{ and if } P_1 \text{ is } >P_2 \tag{5}$$

$$P_3 = P_2 \text{ if } r = 1 \text{ and if } P_1 \text{ is } <P_2 \tag{6}$$

$$P_3 = P_1 + P_2\ (1-P_1) \text{ if } r = 0 \tag{7}$$

$$P_3 = P_1 + P_2 \text{ if } r = -1 \text{ and } P_3 \text{ is } \leq 1 \tag{8}$$

More generalized mathematic models for this form of joint action have been given by Plackett and Hewlett (1948). In the analysis of dose-mortality data, independent action is characterized by a curvilinear log dose-probit response line, which is skewed upward as the incidence of response increases and the correlation coefficient decreases (Finney, 1971; Muska and Weber, 1977).

Differences in expected response based on different types of additivity assumptions can be substantial. For example, going back to Equations 1 and 2, doses associated with response rates of 20% (4.16 probits) are 19.3 and 9.7 for toxicant-1 and toxicant-2, respectively. Under the assumption of dose addition, the expected response of these two doses combined is 5.06 probits or ~53% (see Equation 3). However, assuming response addition, the expected responses for the combination of doses are 20, 36 and 40% for values of r of 1, 0 and -1, respectively.

Several models for toxicant interaction have been summarized by Finney (1971). In addition, Durkin (1981) has proposed a model to deal with the type of asymmetric interaction encountered by Alstott et al. (1973).

However, as illustrated in the following section of this presentation, most of the available data on toxicant interactions are not adequate to test the hypothesis of additivity and cannot be used to estimate the necessary parameters in interactive models.

Measurements of Toxicant Interactions

Approaches to the analysis of toxicant interactions used by most toxicologists have been based on the assumption of dose addition. One common measurement, referred to here as the ratio of interaction (R.I.), is the ratio of the observed EC_{50} of a mixture to the EC_{50} predicted by Equation 3 for dose addition. Most applications of this ratio are based on a single mixture and use questionable methods to determine significance. Keplinger and Deichman (1967) used the ratio of interaction to measure the joint action of various pesticides in mice. In this study, only one mixture of each combination was used, and significant interaction was arbitrarily defined as ratios of 0.57 and less for synergism and 1.75 and greater for antagonism. Smyth et al. (1969, 1970) used a slightly modified expression of the ratio of interaction, which resulted in estimates that approximated a normal distribution. Significant interaction was then defined as those ratios which were beyond 1.96 standard deviations from the mean ratio. In studies on the joint action of pesticides in houseflies, Sun and Johnson (1960) defined the cotoxicity coefficient as the ratio of interaction multiplied by 100. Again, the investigators used only a single mixture. Significant interaction was estimated by taking repeated measurements and determining if the 95% confidence interval of the cotoxicity coefficients included zero. More recently, Wolfenbarger (1973) used cotoxicity coefficients to estimate the joint action of toxaphene-DDT mixtures in insects.

Although different mixtures of this combination were used, no attempt was made to integrate the results into a clear pattern of interaction. In addition, Wolfenbarger (1973) used 95% confidence intervals of the LC_{50} to determine if the mixtures were significantly more or less toxic than either of the components.

Along with these uses of the ratio of interaction, Ohsawa et al. (1975) used dose addition in an attempt to account for the toxicity of technical grade toxaphene based on the toxicity of various toxaphene fractions to houseflies. This concept has enjoyed widespread use in aquatic toxicology (Esvelt et al., 1971). Marking and Dawson (1975) have recently proposed a modified approach in a test for interaction. Like most of the approaches using the ratio of interaction, this method utilizes only a single mixture of each combination. In this method, significance is determined by using the 95% confidence intervals of the LC_{50} of the mixture and two components to estimate the confidence intervals of the additive index. Intervals of the additive index which do not include zero are considered indicative of significant interaction.

All of the above approaches are severely limited by their reliance on a single interactive ratio. As discussed by Hewlett (1969), the ratio of interaction is characteristic only of a particular mixture of a combination. In other words, the estimated value of the ratio of interaction will vary depending on the proportions of the toxicants present in the mixture. This concept is explicitly defined in Equation 3 by the terms f_1 and f_2.

Another limitation in the use of ratios of interaction is encountered in attempts to demonstrate statistical significance. The method used by Sun and Johnson (1960), based on repeated measurements of the ratio of interaction, may be the least objectionable. However, because of the dependence of the ratio of interaction on f_1 and f_2, the estimate of interaction is valid only for the particular mixture tested and has no merit in assessing the overall interaction characteristic of the combination being tested. This limitation may be particularly misleading for those compounds which evidence asymmetric interaction. The approach adopted by Keplinger and Deichman (1967) is totally arbitrary and makes no attempt to establish a criterion for statistical significance. The method of Smyth et al. (1969, 1970) is based on arbitrary selection of test chemicals which influence the criteria for interaction. The other methods which use 95% confidence intervals of the LD_{50} of the mixture and individual components (Marking and Dawson, 1975; Wolfenberger, 1973) are overly sensitive to both endogenous and exogenous variance. Marking and Dawson (1975) recognized the difficulty with exogenous variance in stating that "well-planned toxicity tests which result in narrow confidence intervals are most useful in the assignment of the effects of chemical mixtures." However, if endogenous variation is high (i.e., the slope of the log dose-probit response line is low), even well-designed toxicity tests may yield 95% confidence intervals which preclude the detection of interaction.

The difficulty in demonstrating significant interaction with any of these tests using single ratios of interaction is primarily one of experimental design. Since the ratio of interaction is dependent on the proportions of the components in the mixture, a test has the best chance of demonstrating significant interaction if the mixture giving maximum interaction is selected. If the combination of toxicants being tested is assumed to evidence a pattern of symmetric interaction, a mixture of equitoxic doses would be the best selection. Even with this simplifying and not necessarily valid assumption, however, tests based on single ratios of interaction will not yield significant results unless the magnitude of the interaction is substantial and the experimental variability is minimal.

Possible Approaches Based on Additivity

Two types of approaches may be used by the Agency, depending on whether ADI's or practical thresholds for the different toxicants have been established, or whether dose-response estimates have been made.

In the former case, one approach would be to use a modification of the equivalent exposure index defined by OSHA (37 FR 23502-23505) and recommended by De Rosa (1981) and ECAO (U.S. EPA, 1981). Using this method, a hazard index (HI) for a single toxicant to which individuals are exposed by oral (O), inhalation (I), and dermal (D) routes can be defined as:

$$HI = E_O/Th_O + E_I/Th_I + E_D/Th_D \qquad (9)$$

where E_O, E_I, and E_D are the daily exposures to the toxicant from oral, inhalation and dermal routes, respectively, and Th_O, Th_I and Th_D are the corresponding route-specific practical thresholds. If the hazard index for the compound is less than unity, no hazard is assumed to exist. If the hazard index is greater than unity, a hazard is assumed, but the magnitude of the hazard is defined only in relative terms with respect to the practical thresholds. Although this approach does not define dose-response relationships, it would be possible, if sufficient data were available, to derive practical thresholds for a spectrum of effects (e.g., MFO induction, minimal effects on several organs, severe effects on several organs, reproductive dysfunction, behavioral effects, and mortality). If practical thresholds could be derived for such a spectrum of effects, the hazard assessment could suggest not only if effects were likely to be seen, but also what types of effect, if any, might be expected.

For hazardous waste sites, which would probably involve exposures to more than one toxicant, the total hazard index (HI_T) for the site could be calculated as the sum of the hazard indices for the n number of toxicants of concern:

$$HI_T = HI_1 + HI_2 + \ldots HI_n \tag{10}$$

Again, if practical thresholds for a spectrum of effects could be defined, total site-specific hazard indices could be calculated for each effect.

A multiplication factor for the total hazard index could be recommended if data suggested that several of the toxicants at a site evidenced synergistic effects when applied in combination. For instance, for a site with 10 toxicants, 5 of which were reported to evidence synergistic interaction on liver toxicity, the base total hazard index for liver toxicity could be multiplied by 1.5 (i.e., 0.1 for each of the interacting toxicants); however, such an approach would have only a pretense of predictability. The approach might have merit for "protection", but its use would be a matter of policy, not science, and would ignore the realities and complexities of toxicant interactions. Furthermore, much of the literature reporting "synergism" or "antagonism" makes no meaningful attempt to determine if the observed responses reflect true interaction or simply additivity. Consequently, the decision to use such correction factors for interaction would have to be carefully monitored.

If dose-response relationships have been defined for the individual compounds at each site, information will be available on the expected incidence of response, P, for each effect of concern caused by each chemical for a single route of exposure. If more than one route of exposure is involved, route-to-route extrapolations, combined with the monitoring data/exposure estimates for each route, will be used to calculate the cumulative (i.e., from all routes) expected incidence of response, P, for each effect of concern caused by each chemical. An example of such a data set is given in Table 16, in which five hypothetical chemicals (I to V) are associated with a total of six effects of concern (A to F). The problem is to estimate the expected incidence of response for each effect and the cumulative incidence of adverse response in the population.

Accepting the premise that some form of additivity must be used, the most reasonable approach would seem to be response addition, in which the correlation of individual responses within the population is assumed to be zero. As indicated in Equation 7, the formula for predicting the total expected response (P_T) for exposure to two chemicals, using this assumption, can be expressed as: $P_T = P_1 + P_2\ (1-P_1)$. This equation can be generalized, for any number of chemicals, as:

$$P_T = 1 - \Pi(1-P_i) \tag{11}$$

Using this equation, the cumulative incidence of all adverse responses from each chemical (PC_i) is given in the last column of Table 16 and the cumulative incidence of each adverse effect caused by the combination of chemicals (PE_i) is given in the last row of Table 16.

The calculation of PE_i is a straightforward use of the above equation. The calculation of PC_i, the total incidence of adverse responses caused by each chemical, is somewhat different in that the assumption is that the effects induced by a given chemical are independent of one another. For some combinations of effects [e.g., increased liver weight, MFO induction, proliferation of smooth endoplasmic reticulum (sER) in liver cells] this assumption obviously will be invalid. For such cases, it may be more reasonable to assume that the correlation of tolerances is unity. The implications of this assumption are discussed below.

TABLE 16

Example of Risk Assessment for Multiple Toxicant Effects*

	Effects of Concern						
Chemical	A	B	C	D	E	F	PC_i
I	2×10^{-2}			8×10^{-4}			2.08×10^{-2}
II		3×10^{-3}			1×10^{-3}		4.00×10^{-3}
III			4×10^{-2}			7×10^{-3}	4.67×10^{-2}
IV	5×10^{-3}			9×10^{-3}			1.39×10^{-2}
V		6×10^{-4}				6×10^{-3}	6.00×10^{-4}
PE_i	2.49×10^{-2}	3.60×10^{-3}	4×10^{-2}	9.79×10^{-3}	1×10^{-3}	1.30×10^{-2}	$PT = 7.8\times10^{-2}$

*See text for explanation of terms.

Accepting for the moment that the responses of concern have been selected so that the assumption of independence among responses is reasonable, the total cumulative incidence of all adverse effects from all chemicals (P_T) can be calculated from Equation 11, substituting PE_i for P_i.

At least two major concerns can be expressed about the application of this method: the assumption of tolerance correlation and the combining of responses. As previously discussed in the section "Mathematical Models for Joint Action", several types of response addition are possible, depending on the correlation of individual tolerances (r) within the population. However, the true correlation of individual tolerances to toxicants within the human population is not known. Some evidence suggests that cancer susceptibility in humans may be partially genetic. Furthermore, strain differences within a species in the susceptibility to chemical carcinogens also suggest a genetic component. Thus, a case probably could be made for assuming that r is positive for carcinogens. Nonetheless, the degree of the correlation cannot be estimated and r probably varies for different carcinogens and systemic toxicants. Consequently, it seems reasonable to assume that r equals zero. This can be criticized as being somewhat conservative, but it is certainly less conservative than assuming that r equals -1. Assuming that r equals +1 would probably underestimate the risk. Consequently, Equation 11 is recommended for calculating the total expected response for exposure to multiple carcinogens or systemic toxicants.

It may be of some use to examine the practical significance of assuming $r = 0$ compared to the more conservative assumption of $r = -1$. For the hypothetical data given in Table 16, P_T is 7.8×10^{-2}, assuming $r = 0$. If the assumption was made that $r = -1$, P_T would equal ΣP_i or 9.24×10^{-2} which is ~18% greater than the estimated response assuming $r = 0$

(7.8×10^{-2}). It can be stated that the higher the expected incidence of response, the greater the difference will be between the estimates of response based on the assumption of $r = 0$ and $r = -1$. For instance, if effects A, B, C were considered, the predicted incidence of response would be 6.72×10^{-2} and 6.82×10^{-2} for $r = 0$ and $r = -1$, respectively, a difference of only ~2%. Conversely, if all of the P_1's in Table 16 were increased by a factor of 10, the P_T's would be 6.57×10^{-1} and 9.24×10^{-1} for $r = 0$ and $r = -1$, respectively, a difference of ~41%. This is substantially higher than the 18% difference noted at the original response rates given in Table 16. For most hazardous waste disposal facilities, the P_T probably will not exceed 1×10^{-2}, and differences between the two assumptions should be small. Thus, since the increased conservatism of $r = -1$ will in most cases not be substantial, and since $r = 0$ is a more reasonable biological assumption than $r = -1$, the use of $r = 0$ rather than $r = -1$ seems justified.

However, as indicated previously, it may sometimes be more reasonable to assume that $r = 1$, or at least that r is positive, for some sets of responses. By analogy to the approach used for $r = 0$, P_T could be set at the maximum value of P_1, if the assumption was made that $r = 1$. This could, however, lead to some substantial errors in the estimate of risk. For instance, take the following series of responses in the liver for which r could be assumed to be positive and possibly equal to unity:

	Case 1	Case 2
MFO Induction	0.50	0.5
Proliferation of sER	0.30	0.5
Increased liver weight	0.20	0.4
Liver necrosis	0.05	0.3

Assuming that $r = 1$, the P_T for these effects in combination would be 0.5 for both Case 1 and Case 2, although Case 2 would be of greater concern than Case 1.

The most obvious, and perhaps the most defensible, approach would be not to combine effects. Thus, in Table 16, the PE_i's could be used to estimate the expected incidence for each effect, but no estimate of P_T would be made. If it is necessary to estimate P_T, it will be necessary to segregate the effects into categories, in which the assumption of $r = 0$ is reasonable. For each of these categories, a P_i would then be estimated so that the severity of the effect is comparable to the other effects or categories of effects which are being combined.

Note: Throughout the above discussion, it has been assumed that the effects are quantal or can be treated as quantal responses. It is unlikely that sufficient data will be available on graded responses to allow for a quantitative analysis.

CRITIQUES

DR. THOMAS CLARKSON: BIOLOGICAL BASES

At low levels, the kinds of interactions will possibly be nothing more than the independent action of the individual chemicals because the receptor sites, transport systems, etc., will not be saturated.

The mathematical approach is very complicated and possibly too expensive to use. Maybe the model could be used for statistical prediction as a first screen.

Nearly all our examples are from pharmacology and deal with rapidly acting drugs that cause acute effects. It is important to study examples of interactions among environmentally important agents.

• Tumor promoters	- Cigarette smoking	
• Dioxin receptors	- Dioxins - Dibenzofurans - PCBs - Aryl Hydrocarbons	Toxicity induction of AHH and genetic control
• Antioxidants	- Selenium/CCl_4 Toxicity - Vitamin E/CCl_4 Toxicity - GSH/Carcinogens	

The area of lipid peroxidation involving oxidizing free radicals contains many examples of interactions.

• Intestinal microflora	- toxification of agents, e.g., cycasin - detoxification, e.g., methylmercury - diet -- $T^{1/2}$ methylmercury
• Enterohepatic circulation	- Kepone and exchange rings - Diet -- methylmercury
• Ethanol	- CCl_4 - Hg°
• SO_x, NO_x	- Clearance of deposited aerosols from lung.
• Gastrointestinal absorption	- Ca^{++} and Fe^{++} versus Pb^{++}
• Complexing agents Natural and man-made (F^-)	- Metals - Aluminum Zinc

- Kidney interactions: Loss of epithelial cells leads to increased resistance (F^- versus Pb^{++})
- Induction of metal protein
  - thionein (cadmium)
  - nuclear inclusion bodies (lead)

DR. HERBERT CORNISH: BIOLOGICAL BASES

Toxicant interactions may involve the interaction of a chemical toxicant with a biological component. A typical example is the nonenzymatic reaction of unsym-dimethylhydrazine (UDMH) with vitamin B_6 (pyridoxal phosphate) to form a hydrazone. The resulting acute B_6 deficiency results in hyperexcitability and convulsions in experimental animals. Treatment with pyridoxine results in prompt alleviation of the CNS symptoms.

An interesting phenomenon is where the action of a second toxicant can alter the organ specificity of the first. 1-Nitronaphthalene produces both lung and liver toxicity in normal rats. Pretreatment with phenobarbital prevents the lung damage and enhances hepatotoxicity. This is accompanied by altered rates of excretion of metabolites and altered patterns of covalent binding in the lungs and liver.

Ethanol has long been known as a potentiator of halogenated solvent hepatotoxicity. Several other alcohols, such as methanol, isopropanol, or secondary and tertiary butanols, are also excellent potentiators of toxicity. Recent literature reports show that not only isopropanol, but also its metabolite acetone potentiate halogenated solvent toxicity. Further studies with diabetic rats demonstrate that the uncontrolled diabetic rat was at greater risk from carbon tetrachloride exposure than was the normal rat. Thus diabetics may be a susceptible subgroup in the human population.

Of major concern is how to make the best use of known information on synergism or antagonism in risk assessment of a specific mixed exposure situation. It appears relatively easy to add another safety factor when synergistic effects of two chemicals have been demonstrated. Are we equally comfortable in making a similar type of judgment when antagonistic effects of two chemicals have been amply demonstrated?

DR. KENNETH CRUMP: MATHEMATIC MODELS

The proposal to use a mathematical model to estimate a "benchmark intake" for setting ADIs seemed to me to meet with a generally favorable reaction. The principal objection was the lack of the necessary quantitative data for some toxic endpoints. This would mean that the methodology could not be applied universally, although it could be used in many instances. I believe that adoption of such an approach could have the useful side benefit of promoting a greater degree of quantification and improved data-reporting procedures in toxicological studies. To achieve the greatest impact in this area, the methodology should be presented in the scientific literature. Details of the method that need to be worked out and agreed upon include:

- Definition of benchmark (e.g., dose corresponding to 10^{-1} risk).
- Mathematical model to be used in setting benchmark.
- Whether confidence limits should be used in setting the benchmark (my own answer to this is definitely yes).
- How to combine data for different toxic effects (e.g., whether to use different safety factors to reflect severity of effects).

The suggestion I made in my discussion for setting ADIs for mixtures is as follows: Let f_i be the fraction of the mixture made up of chemical i and let ADI_i be the ADI for chemical i. Assume the risk from each chemical is linear in dose, i.e., that risk from exposure to chemical i alone is given by

$$R = q_i \text{ dose.}$$

If we assume all ADIs are comparable in the sense that they all correspond to the same risk R, then it follows that

$$ADI_i = R/q_i.$$

Now assume further that the risk from exposure to a mixture is the sum of the risks from the component chemicals. Then if $ADI_{mixture}$ is also to correspond to risk R, it must satisfy

$$R_1 + . . . + R_k = R$$

where R_i is the risk from the ith chemical. But this can be written as

$$q_1 f_1 ADI_{mixture} + . . . + q_k f_k ADI_{mixture} = R$$

or

$$ADI_{mixture} = \frac{R}{q_1 f_1 + . . . + q_k f_k} = (f_i/ADI_1 + . . . + f_k/ADI_k)^{-1}$$

This approach is consistent with additivity in risk and linearity in dose. It could be used with mixture of carcinogens and systemic toxicants. It seems inevitable that some form of additivity must be the basis for estimating allowable exposures to mixtures. A reasonable argument can be made for such an approach, and data needed for more complicated methods will almost never be available.

DR. MARVIN SCHNEIDERMAN: MATHEMATIC MODELS

There is a thoughtful (and useful) publication by the NAS/NRC (1980) on the problems of joint toxicity. The work was done under the chairmanship of Dr. Sheldon Murphy of the Department of Pharmacology of the University of

Texas on the behalf of the Coast Guard. Why the Coast Guard? Coast Guard offices inspect ships, including the tanks and holds in which chemicals are shipped, and are thus exposed to many toxic materials, sometimes in combination, more often in sequence. The Coast Guard was concerned with long range effects. Chapter 9 and Appendix B of the report discuss some of the mathematical models of joint or combination actions. Dr. John Gart of the National Cancer Institute wrote Appendix B.

An operational conclusion of the Coast Guard report is that for relatively low exposures, a good first approximation to overall toxicity can be made by (effectively) adding the toxicities. For materials treated as if they were linear in dose-response and without threshold, additivity in response is equivalent to additivity in dose, and there is no need to make distinctions between which of the additive models will be used.

For materials for which a threshold is assumed (or presumed), additivity in response can be in error, and additivity in dose is the only appropriate approach. An example should make this obvious. Say we have a "threshold" material, and the threshold is at 5 units of dose. At any lower dose, response is zero. Say this material exists in the ambient environment (Source A) at a level of 3 units. There will be no toxic responses. It also exists in the elutions from a toxic waste dump (Source B) at a level of 3 units. Persons exposed only to the toxic dump product will show no toxic responses. When we have persons exposed to both sources we could say (using additivity in response) that persons exposed to Source A should show no response; persons exposed to B show no response; hence persons exposed to A and B should obviously show no response. Zero + zero = 0. But Source A plus Source B gives 6 units of dose, and the threshold was 5. Hence, there should be response. Adding doses leads to the conclusion that there should be response after exposure to both A and B.

The major problem with trying to do something with adding doses is that we usually need to know the dose of the active material at the site of the action. Nominal dose (of different materials) is very hard to convert to "real" dose. This problem is exacerbated by the fact that standards usually need to be set in terms of nominal doses.

Whether combinations of materials act additively (in any sense) or more than additively (combining results of response to Source A and Source B, if we didn't know these came from the same material, would lead us to say -- using additivity in response, as is usually done -- that A and B were synergistic) is usually rather hard to determine; and once we determine it in the mouse or the rat, we still cannot be certain that mouse synergism (or antagonism) will also be human synergism (or antagonism).

It may be helpful to look at some past attempts at uncovering possible synergism to see if these provide useful suggestions for possible experimental evaluation of the toxicity of combinations of materials that find, or might find, their way out of toxic waste dumps. Figure 28 shows schematically one of the approaches by Abraham Goldin and Nathan Mantel (Goldin et al., 1958, 1974) in their (successful) attempts to find combinations of chemotherapeutic drugs to be used against human leukemia. Mantel speaks of this as attempting to find "therapeutic synergism." The model system they used was the L-1210 mouse leukemia -- a transplanted leukemia that kills rather quickly. The figure is an attempt to show in three dimensions the separate effects of two materials in increasing the lifespan of L-1210 bearing mice.

At zero dose the mice have the survival of the control animals. At increasing dose (assuming the material is effective) there will be increasing survival until the toxicity of the drug begins to intervene and survival

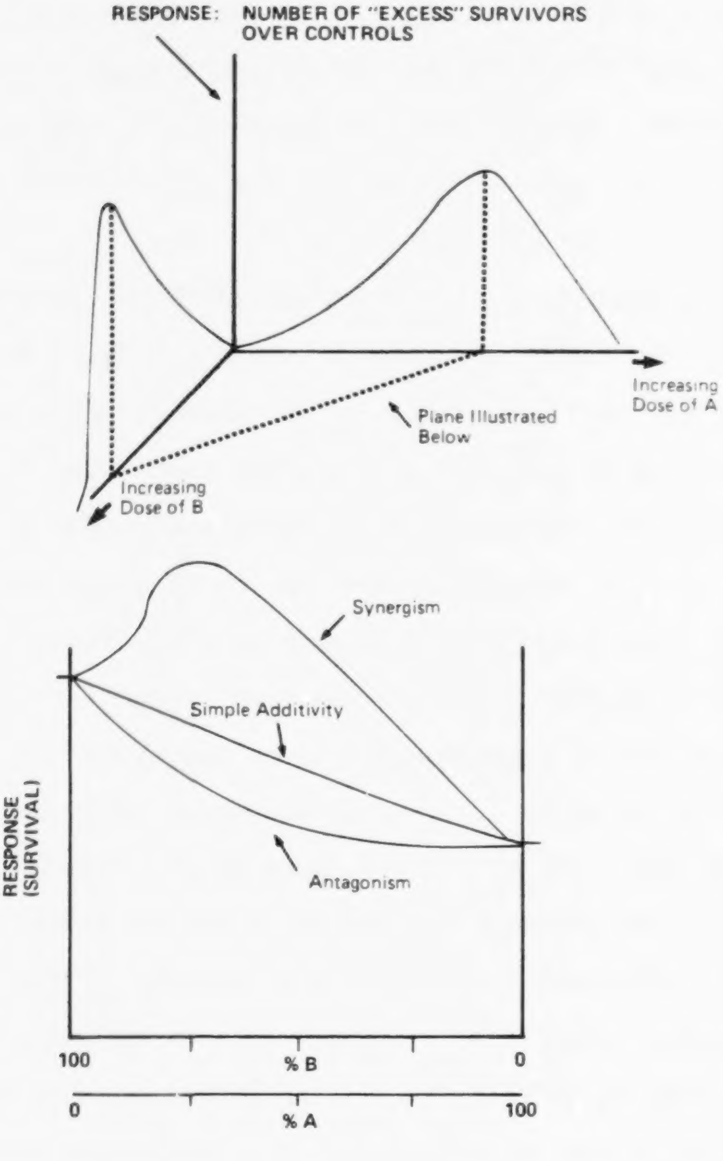

RESPONSE: NUMBER OF "EXCESS" SURVIVORS OVER CONTROLS
Increasing Dose of A
Plane Illustrated Below
Increasing Dose of B
Synergism
Simple Additivity
Antagonism
RESPONSE (SURVIVAL)
100
% B
0
0
% A
100

FIGURE 28

Conceptual dose-response relationships for two chemotherapeutic drugs. (A) Three-dimensional representation of separate effects of materials A and B. (B) Vertical plane through doses of A and B that elicited maximum response. Possible synergistic, antagonistic and simple additive dose-response curves for combined chemotherapy are shown.

Source: Adapted from Golden et al., 1958, 1974 is then reduced from the peak. The Goldin-Mantel procedure was to take the optimal dose of one material (say A) and the optimal dose of another material (say B), and create an array of pseudomaterials (AB) consisting of different proportions of A and B. The trace of these materials on the base plans (in the figure) can be thought of as rays in the horizontal plane starting at the (0,0) point. Each ray might be considered as one combination of A and B (e.g., say 30% A and 70% B). The farther one moves on the ray from the (0,0) point, the higher the dose.

The bottom part of Figure 28 shows a plane taken out of the three-dimensional representation of the top part of the figure where the best responses were shown for the individual materials. At the left end of this plane we have 100% B and, on the Y axis, the response (survival) at this dose. At the right end we have 100% A and, on the Y axis, the survival at this dose of A. The next stage of experimentation would be to set up an array of different pseudomaterials, each material corresponding to different proportions of A and B, and conduct survival (dose-response) experiments at several dose levels. The responses will create a "mountain" rising out of the base plane. The bottom of Figure 28 shows a slice taken through such a mountain, with three possible contours. The top contour shows a response of A and B that will yield a higher response than the best of A and B alone. The optimal, as the figure is drawn, looks as if it comes at about 60% B and 40% A.

The lowest line in the figure shows that the combination of A and B is deleterious, and that the best response would come at 100% B and 0% A. (These two materials are antagonistic!) The middle line shows that A and B just dilute each other, and the best response would also come at 100% B.

If a large experiment had been conducted at several different dose levels of the combination of A and B, it is possible (likely?) that the peak of the mountain would have come somewhere other than along the sample plane drawn here.

All of this looks very time consuming and complicated, and it was. There are some mountain-peak search strategies that have been developed ("maximum-seeking" strategies) that could shorten the process, but all of them required lots of work and rather prompt response. In the chemotherapy problem, very few materials needed to be looked at, and the experiments usually took no more than 30-45 days from beginning to burning (of the dead mice in the incinerator), so that this search process was possible and did prove effective.

Figure 29 shows how a Goldin-Mantel scheme might look if one were looking for joint toxicities. The bottom part of the figure shows the plane that includes the ED_{50}s or LD_{50}s of the two materials. If some dosage combination of A and B produces more toxicity (at equivalent total dose), then the toxicities would be more than additive, as in the Goldin-Mantel model. There are many added complications, however. How do we combine different toxicities? If there are several effects what do we do with them? If it takes a long time to get an answer (i.e., chronic illness; lifespan measures; long, latent period illnesses like cancer), is it even possible to go through a Goldin-Mantel process? And, finally, if there are a lot of materials to consider jointly, is it possible to do even a pairwise Goldin-Mantel procedure?

Let us determine the magnitude of the combinations of materials that might come about when there are several materials that might leak out of a toxic waste dump, either one at a time or in any combination with each

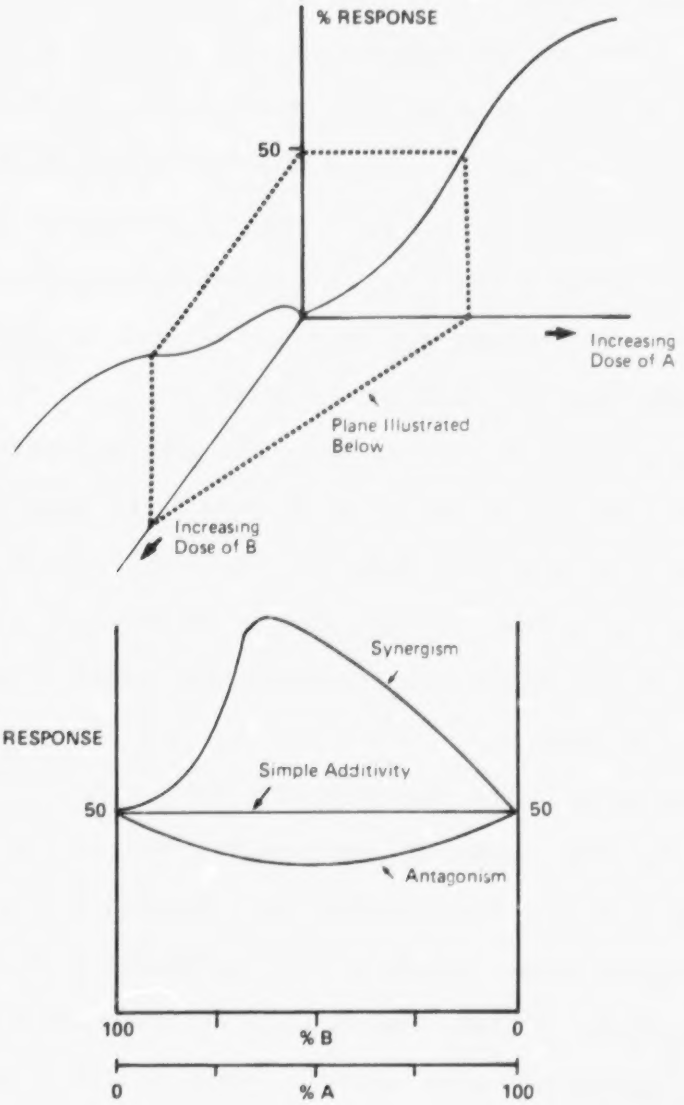

% RESPONSE
50
Increasing Dose of A
Plane Illustrated Below
Increasing Dose of B
Synergism
% RESPONSE
Simple Additivity
50
50
Antagonism
100
% B
0
0
% A
100

FIGURE 29

Conceptual dose-response relationships for two toxic substances. (A) Three-dimensional representation of separate effects of materials A and B. (B) Vertical plane through doses of A and B that elicited 50% response. Possible synergistic, antagonistic and simple additive dose-response relationships are shown.

Source: Adapted from Golden et al., 1958, 1974 other. Rather than consider different proportions of each of the materials, we will calculate the combinations possible for just presence or absence of the material. Thus, two materials can be present in an effluent in three ways (e.g., A alone, B alone, or A and B together); three materials in seven wasy (e.g., A or B or C, AB, AC, BC or ABC). The general form is $2^n - 1$. Ten materials could be present in $2^n - 1 = 1023$ combinations; and this large number does not consider the materials present in different proportions, only whether they are there or not there.

Thus, it seems clear that it is most unlikely that direct measures of the joint toxicities of the multitude of materials found in toxic waste dumps can or will be made. In addition, it seems reasonable to consider further possible interactions of toxic waste dump materials with other materials in common use or those to which people are commonly exposed, such as ethanol or cigarette smoke.

Clearly, approaches other than detailed, definitive testing need to be developed. It has been suggested, for example, that as a first approximation, joint tests of 1,1,1-trichloroethylene, the most commonly found material in toxic waste dumps, be tested in combination with other commonly prevalent materials -- or that commonly prevalent toxic waste dump materials be tested in combination with ethanol. None of these suggestions, of course, is completely satisfactory.

Not much combination testing for carcinogenesis (which seems to be the major basis for standard setting for single materials) has been done in the past. The testing in the large NCI-Stanford Research Institute combination experiment (24 pairwise contrasts) has been completed for some time, but analysis is not complete. So far as I know, no substantive publications have come from this study, but some are in the process. Several years ago, when I had an opportunity to look at some preliminary data, I could make no generalizations. I found some combinations that appeared additive or perhaps more than additive if the cancers they produced had the same target tissues. When the carcinogens affected different target tissues their actions often appeared to be less than additive, largely because the animals often died early from the first cancer and did not have an opportunity to develop tumors at the other, later-appearing site. The analysis of these data is likely to be very difficult, requiring consideration of competing causes of death, growth and weight gain, and allowing for time to tumor appearance.

DISCUSSION

DR. PATRICK DURKIN

As Drs. Clarkson and Cornish recommended, a major effort must be made to identify and explicate examples of environmentally significant multiple toxicant exposures and their related effects.

Another major area to explore, as I indicated in my presentation, is the potential low-dose significance of the interactions. I suggested, based on my understanding of the biological modes by which chemicals interact, that in the low-dose region the interactions may be quantitatively less significant or may not occur. Dr. Crump reinforced this feeling in his statistical analysis. I think Dr. Crump's approach should be explored in greater depth. In addition, I am examining multiple-order models along the same lines as an extension of the earlier work by Hewlett (Hewlett, 1969; Hewlett and Plackett, 1950, 1959; Plackett and Hewlett, 1948, 1952). Also along these lines, I am examining examples of subchronic studies that involved multiple toxicant exposures at relatively low doses.

An additional point that was not extensively discussed at the meeting involves the quantitative significance of multiple toxicant exposures. Over the next month or so, I will be addressing this issue for Dr. Stara's office. Although most of the data will probably come from acute studies, I will make every effort to examine the quantitative significance of interactions in the low-dose region.

MR. WILLIAM GULLEDGE

Definition of the term "synergism potentiation" is unclear as it was presented at the workshop. If synergism is defined as something less than additive effects, this definition is favorable. Very little evidence exists to indicate that chemicals act by additive effects. Dose-addition experiments have shown that chemicals act independently of one another in terms of observable effect. Any argument for toxic interaction stands on weak ground unless compounds are naturally reactive in some manner.

DR. MAGNUS PISCATOR

The whole section on interactions, well written and theoretically good, applies to large doses of toxic agents and may have little relevance to what happens at low level exposure.

DR. ROLF HARTUNG

The assumption has often been made that the toxicity of chemicals is likely to be additive, and it is hoped that potentiating responses will be offset on the average by antagonistic responses. Whether any of these interactions actually occur at low doses under chronic conditions has not been satisfactorily established. The frequently cited examples of the interactions between asbestos and cigarette smoke are probably not typical examples, since they probably involve the interactions of initiation-promotion processes, and also tend to involve relatively high doses. It is important that interaction studies, such as the 16-compound NCI/SRI study be published for evaluation. It is also important to study the effects of ongoing multiple exposure (e.g., diet), and to set up experiments on multi-component mixtures in order to test interactions in general principle.

DR. RICHARD KOCIBA

These discussions underscored the premise that one cannot categorically assume that all chemical interactions should be treated as synergistic or additive phenomena. There were numerous cited examples wherein direct chemical-chemical interactions usually led to a decrease in toxicologic activity rather than a synergistic or additive effect.

It appears as if there is little scientific justification to categorically assume that multichemical exposure warrants the assumption of synergism or additivity unless indicated by available data. A case-by-case approach would appear to be the most appropriate course of action in dealing with these multichemical exposures. This would best utilize all the data available on the chemicals comprising the multichemical exposure.

DR. HERBERT CORNISH

At the moment there is little basis for assuming other than an additive effect of chemicals at any one site.

DR. THOMAS CLARKSON

The two models described by Dr. Durkin refer only to interaction with receptors; no modes are available to deal with pharmacokinetic interactions. If one chemical produces serious tissue damage, effects on the pharmacokinetics and/or toxicity of a second chemical might be expected. However, the addition of responses is reasonable for low exposure levels. Chemicals would be expected to act independently and not to interfere with either their respective pharmacokinetics or with the reaction with their respective receptors.

Dose addition is also reasonable for these chemicals acting on the same receptor. Saturation of the receptor is unlikely.

DR. ROBERT NEAL

In calculating the allowable exposure of man to compounds that produce cancer in experimental animals, mathematic extrapolation is the best technique currently available. However, we should not delude ourselves that we have a biological basis for extrapolating from the observable range in experimental animals to the low levels to which man is normally exposed. At this time there is clearly no justification for sophisticated manipulations of mathematical models to estimate what the incidence rate may be in man, based on incidence rates at high doses in experimental animals. At best, the data generated using the mathematic models are a guess.

Since there is no biologic basis for choosing between the various mathematical models used in extrapolating from high-dose animal experiments to low-dose human exposure, there is some merit in standardizing the mathematic model used by regulatory agencies in estimating cancer risk. However, in applying a standardized model, consideration should be given to different levels of allowable risk depending upon the applicability of the cancer data ("weight of evidence") generated in experimental animals to man. For example, a compound that causes a tumor incidence in only one sex of one species and not in the other sex of that species or in other species examined should perhaps be given less weight than a compound that produces tumors in multiple sites and multiple species.

An approach to considering the weight of evidence in terms of risk assessment for carcinogenicity has recently been proposed by Dr. Robert Squire in an article in Science (Squire, 1981). However, the method proposed by Dr. Squire does not provide a numerical estimate of risk that can be used by the regulator in setting an allowable level of exposure. A modification of the Squire proposal might be to accept a higher level of risk, determined by mathematic extrapolation, for those compounds for which the weight of the evidence is such that there is some question as to the applicability of the data generated in experimental animals to man. For example, an allowable risk of 1 in 10,000 might be allowed for a compound that only increases the incidence of liver tumors in male mice but not in female mice or in rats, whereas an allowable risk of 1 in 1,000,000 be considered for a compound that causes tumors in multiple organs of multiple species. Option 3 proposed by Dr. Crump is also a modification of that same concept.

DR. WILLIAM NICHOLSON

For combinations of exposures at low doses, each with low risks (10^{-4}, etc.), additivity of effects would appear to be justified. This would apply both for carcinogenic agents and those demonstrating only systemic effects. However, additivity may not apply when the exposure to an agent in a dump site or in water combines with a personal exposure to an agent that can be significant. Such personal exposures include 1) cigarette smoking, 2) alcohol consumption, 3) medicinal or other drug use, and 4) special unique exposure circumstances. Here the effects may be directly multiplicative, especially for lung carcinogens. Thus it would be important to establish some of the combined effects for the dozen or fewer chemicals of concern in the environment and the agents to which some humans could have extremely high exposures. Where a review of the literature indicates that such data are lacking, appropriate research should be undertaken.

DR. MYRON MEHLMAN

Insufficient data are available to develop any meaningful model without further consideration of biologic processes.

MR. WILLIAM GULLEDGE

Due to the uncertainty in predicting low doses and the various options associated with the multistage model, recommendation for uniform use in establishing water quality criteria cannot be given at this time. It would seem that most models are equally predictive to a risk level of 10^{-2}, and a combination of mathematic model and safety factors would lessen the necessity for choosing one model over another.

Hazard assessment indices tend to magnify problems of uncertainty. Most carcinogenesis indices take into account only positive test data. Data that would indicate that a material is probably not carcinogenic also need to be considered. Frequently, technical studies are contradictory as to the carcinogenic potential of a substance. The negative data also should be considered as well as differentiation of poor quality studies and very definitive studies.

DR. IAN NISBET

I was disappointed that the second day of the workshop was devoted almost entirely to 2-chemical interactions, since we live in an N-chemical world. I liked Dr. Crump's derivation of the conclusion that interactions are unimportant to first order, although I think it only applies when effects of all N chemicals are small. My quick extension of his result to second order was incorrect, I am afraid, but I will try to develop a correct version.

GENERAL COMMENTS

- The interactions of the different compounds in the dump sites may not be as significant as the interactions of these compounds with smoking or alcohol use.
- Dose additivity for first-order interactions should hold for second order as well.
- Biologic aspects should not be oversimplified with hypothetical examples.
- A dose-response cannot be determined for the mixture due to problems with extrapolation and because the composition of the mixture would vary from place to place and time to time.
- Interactions are species-specific.
- The RCRA weighting scheme should be considered.
- If we have a sufficiently good data set over a wide range of chemicals, we may be able to predict risk from multichemical exposure.

REFERENCES

Alstott, R.L., M.E. Tarrant and R.B. Forney. 1973. The acute toxicities of 1-methylxanthine, ethanol, and 1-methylxanthine/ethanol combinations in the mouse. Toxicol. Appl. Pharmacol. 24: 393-404.

Bliss, C.I. 1939. The toxicity of poisons applied jointly. Ann. Appl. Biol. 26: 585-615.

De Rosa, C. 1981. U.S. EPA, Cincinnati. Memorandum to Jerry Stara, U.S. EPA, Cincinnati, July 23.

Durkin, P.R. 1981. An approach to the analysis of toxicant interactions in the aquatic environment. In: Proc. Fourth Ann. Symp. on Aquatic Toxicology. Am. Soc. Test. Mater.

Esvelt, L.A., W.J. Kaufman and R.E. Selleck. 1971. Toxicity Removal for Municipal Wastewaters, Vol. IV, of a Study of Toxicity and Biostimulation in San Francisco Bay-Delta Waters, SERL Report No. 71-7, Univ. of California, Berkeley, CA. p. 224.

Finney, D.J. 1971. Probit Analysis. 3rd ed. Cambridge Univ. Press, Cambridge, Great Britain. 333 p.

Goldin, A., S.R. Humphries, J.M. Venditti and N. Mantel. 1958. Factors influencing antitumor synergism: Relation to screening methodology. Ann. NY Acad. Sci. 76: 932-938.

Goldin, A., J.M. Venditti and N. Mantel. 1974. Combination chemotherapy: Basic considerations. In: Handbook of Experimental Pharmacology, A.C. Sartorelli and D.G. Johns, Ed. New Series XXXVIII/1. Springer-Verlag, Berlin. p. 411-448.

Goldstein, A., L. Aronow and S.M. Kalman. 1974. Principles of Drug Action: The Basis of Pharmacology, 2nd ed. John Wiley and Sons, Inc., NY. 854 p.

Hewlett, P.S. 1969. Measurement of the potencies of drug mixtures. Biometrics. 25: 477-487.

Hewlett, P.S. and R.L. Plackett. 1950. Statistical aspects of the independent joint action of poisons, particularly insecticides. II. Examination of data for agreement with the hypothesis. Ann. Appl. Biol. 37: 527-552.

Hewlett, P.S. and R.L. Plackett. 1959. A unified theory for quantal response to mixtures of drugs: Non-interactive action. Biometrics. 15(4): 591-610.

Keplinger, M.L. and W.B. Deichmann. 1967. Acute toxicity of combinations of pesticides. Toxicol. Appl. Pharmacol. 10(3): 586-595.

Klaassen, C.D. and J. Doull. 1980. Evaluation of safety: Toxicologic evaluation. In: Toxicology: The Basic Science of Poisons, J. Doull, C.D. Klaassen and M.O. Amdur, Ed. Macmillan Publishing Co., Inc., NY. p. 11-27.

Levine, R.E. 1973. Pharmacology: Drug Actions and Reactions. Little, Brown and Company, Boston, MA. 412 p.

Marking, L.L. and V.K. Dawson. 1975. Method for Assessment of Toxicity or Efficacy of Mixtures of Chemicals. USDI, Fish Wildl. Serv., Bur. Sport Fish. Wildl., Washington, DC, Investigations in Fish Control, No. 67, p. 1-8.

Muska, C.F. and L.J. Weber. 1977. An approach for studying the effects of mixtures of environmental toxicants in whole organisms performance. Recent Advances in Fish Toxicology, R.A. Taub, Ed. EPA 600/3-77-085. p. 71-87.

NAS/NRC (National Academy of Sciences/National Research Council). 1980. Principles of Toxicological Interactions Associated with Multiple Chemical Exposures. Prepared by the Panel on Evaluation of Hazards Associated with Maritime Personnel Exposed to Multiple Cargo Vapors. National Academy Press, Washington, DC.

NRC (National Research Council). 1980. Principles of Toxicological Interactions Associated with Multiple Chemical Exposures. Natl. Academy Press, Washington, DC.

Ohsawa, T., J.R. Knox, S. Khalifa and J.E. Casida. 1975. Metabolic dechlorination of toxaphene in rats. J. Agric. Food Chem. 23: 98-106.

Plackett, R.L. and P.S. Hewlett. 1948. Statistical aspects of the independent joint action of poisons. Ann. Appl. Biol. 35: 347-358.

Plackett, R.L. and P.S. Hewlett. 1952. Quantal responses to mixtures of poisons. J. Roy. Stat. Soc. B14(2): 141-163.

Smyth, H.F., C.S. Weil, J.S. West and C.P. Carpenter. 1969. An exploration of joint toxic action. I. Twenty-seven industrial chemicals intubated in rats in all possible pairs. Toxicol. Appl. Pharmacol. 14: 340-347.

Smyth, H.F., C.S. Weil, J.S. West and C.P. Carpenter. 1970. An exploration of joint toxic action. II. Equitoxic versus equivolume mixtures. Toxicol. Appl. Pharmacol. 17: 498-503.

Squire, R.A. 1981. Ranking animal carcinogens: A proposed regulatory approach. Science. 214: 877-880.

Sun, Y-P. and E.R. Johnson. 1960. Analysis of joint action of insecticides against houseflies. J. Econ. Entomol. 53: 887-892.

U.S. EPA. 1981. Guidelines and Methodology for Quantitative Risk/Hazard Assessment of TSPC Solvents. Environmental Criteria and Assessment Office, Cincinnati, OH. (Unpublished report)

Veldstra, H. 1956. Synergism and potentiation with special reference to the combination of structural analogues. Pharmacol. Rev. 8: 339-387.

Weisburger, J.H. and G.M. Williams. 1980. Chemical carcinogens. In: Toxicology: The Basic Science of Poisons, J. Doull, C.D. Klaassen and M.O. Amdur, Ed. Macmillan Publishing Co., Inc., NY. p. 84-138.

Withey, J.R. 1981. Toxicodynamics and biotransformation. In: International Workshop on the Assessment of Multichemical Contamination. Milan, Italy. (Draft)

Wolfenbarger, D.A. 1973. Synergism of toxaphene-DDT mixtures applied topically to the bollworm and the tobacco budworm. J. Econ. Entomol. 66(2): 523-524.

SUMMATION OF MEETING

AND

CONCLUDING COMMENTS

SUMMATION OF MEETING

DR. ROLF HARTUNG

The purpose of this meeting was to examine approaches to risk assessment for multiple chemical exposures. The first day was spent largely looking at the effects of single chemicals on various species. Initially, we discussed the present methodology for derivation of an ADI and reviewed the NOEL and other approaches. We looked at derivations of uncertainty factors, saw what some of the derivations were and how they were utilized, taking into account bioconcentration factors. Then we heard some of the newer methodologies described that included, for instance, adjustments based on body surface area or various uncertainty factors. What was not settled, even though it was pointed out, was to what extent a body surface area adjustment would take the place of some of the uncertainty factors that now substitute for it, or that have previously substituted for it. It was also pointed out that there is probably no scientifically verifiable means of setting an ADI. As a matter of fact, it is only when we fail in our risk assessments and our attempts to protect that we can see what may have gone wrong and analyze the problem. There were also important discussions on approaches to differentiations between safety factors; the numbers that should be used. and when and what uncertainty factors should be added to these numbers; and the consideration of uncertainty of data versus uncertainty in the extrapolation process.

Throughout the early part of the meeting, there were a number of important points brought up involving the increased utilization of pharmacokinetics. However, the exact means of how to do this needs further clarification. Most chemicals found at dump sites have significantly poorer data bases than do the pesticides and food additives for which a large segment of our present NOAEL-based risk assessments were originally developed. So, the question of missing data for some of the industrial chemicals may need to be addressed.

It also became quite clear that we need to verify or increase the data base for quantitative structure-activity relationships. One observation that appeared to be somewhat comforting was that most of the dumps, at least at first sight, contained only several key chemicals, which tended to repeat from dump site to dump site. However, it was pointed out that some of this information may be an artifact of the chemical priority testing schemes used for ease of analysis, and that there may be large groups of chemicals that are missed. A good candidate group for these possibly missed chemicals (which I would like to add) is that of the aromatic amines, which are very recalcitrant as far as analysis is concerned.

One area for which we had relatively little resolution was species difference. The extent to which a mouse is a man, or a man is a mouse, has not really been resolved except to point out specific differences, some of which are almost anecdotal. It is clear that there are differences in size and metabolic rate, and occasionally differences in pharmacokinetics, response, cell turnover, etc. It was pointed out that we probably could use this type of data, especially for some of the better known solvents, more effectively than we have in the past. In trying to make species-to-species conversions, Dr. O'Flaherty pointed out a number of interesting relationships that have been used in aquatic toxicology based on log-log transforms, but she also pointed out that there needs to be a better mechanistic understanding of some of the phenomena that were observed by Sideranko. It was pointed out that there are instances in which the large animal is less sensitive than the small animal, although the rule tends to be that, at least on a mg/kg or mg/m³ basis, the small animal tends to be less sensitive than the large animal. For instance, the assumption that a mouse should be less sensitive to chloroform than a human did not hold up. In this particular case, the mouse is much more sensitive, but this seems to be at least in part an exception to the rule. There may be enough exceptions so that such a rule cannot be readily applied.

The next areas that were discussed involved route-to-route extrapolations. These particular types of extrapolations are still difficult because they depend greatly on pharmacokinetics and our understanding of them. A point was made that a need exists for test systems that have no false negatives; however, a test system having false negatives produces a consumer risk while one having false positives produces a producer risk. The problem may have to be investigated for specific tests, especially with respect to the number or the degree of false negatives that different tests produce in relation to the false positives they produce. Personally, I know of no test system that has only false positives or only false negatives.

There was significant discussion on incidence and severity of effect. The present NOEL approach works with relatively limited data and ignores the slope of dose-response curves. It was mentioned that the NOEL approach looks at only one small portion of the data, but this statement is probably incorrect because, in reality, when a NOEL is derived one looks at the entire data set, looks to the extent to which it represents a coherent whole, and then selects the threshold level. One does not arbitrarily just pick the lowest number that exists. It is correct, however, that it ends up being a point that is based largely on scientific judgment. It is a single point and one cannot extrapolate downwards from it, therefore it cannot be used for assessing lower risk levels. Its applicability has been blessed by tradition. It may have served us well, but there haven't been too many studies that have verified how well it has served in the past. It was pointed out that it may be useful to have the option of using dose-response data and extrapolation models for systemic toxic phenomena; but it was also pointed out that some of the most sensitive types of effects, such as histopathology, as presently reported in the open literature, may not readily lend themselves to that kind of an approach, while the data as they exist in the gray literature, consultant reports, etc., indeed would allow such an analysis. It was indicated that it might be possible to present incidence data in some of the reports, but this would require changes in the habits of pathologists and editors. It was also pointed out that there is a great deal of literature that exists for drugs, which looks at different physiologic phenomena in relationship to pharmacokinetics, and which looks at metabolic processes and has tied them into an operating approach to pharmaceutical research. These approaches have not been fully utilized for the analyses of environmental toxicants and probably could be utilized to a greater extent.

In our discussion of the extrapolation of responses to systemic toxicants or carcinogens, it was pointed out that, outside of the experimental range, one is increasingly dependent on the assumptions of the model that has been selected, and that model selection, especially for systemic toxicology, represents a judgmental process. It is necessary to try and understand whether the best approach might be that of using a linear model, starting from the lowest point that would define the upper bounds for all concave curves, and thereby introduce a system of conservatism. In my opinion, the question as to how far systemic effects should be modeled, handled with safety factors, and handled with extrapolations to various levels like 10^{-2}, 10^{-3}, etc., was left in the air, and probably proposed only at this time as a comparative type of tool.

Route-to-route extrapolation was discussed. The problems with route-to-route extrapolation, especially as related to the Stokinger-Woodward equation, were reviewed. In addition, the importance of the time course of the dose as well as the resulting blood levels were indicated, especially when ingested doses were to be compared with inhaled doses. The effect of the half-life of a chemical was considered as particularly important, and appears, in some cases, to alter some of the influences of the delivery rate of individual doses. It was also pointed out that even though one may be able to calculate an equivalent absorbed dose by various routes, one would still also need to take into account site-specific effects at the portal of entry, which may indeed greatly influence the overall toxicity.

The question of simultaneous multiple route exposures is a difficult one. The way this has been treated in the past is to observe the proportional absorption from each particular route in order to calculate total body dose. A number of semipolitical problems were pointed out, such as that of trying to add partial doses related to ADI to partial doses related to a TLV, which was judged not to be appropriate. Problems were discussed with the present approach of subtracting a dietary intake and an inhalation dose from the ADI so as to apportion the controllable levels due to water intake alone. Although it is true that the doses should be apportioned, it may require a more complex or a more integrated scheme of cooperation of different units within the Agency.

We shifted to a discussion of the risk assessments for carcinogens. Dr. McGaughy pointed out a two-stage type of evaluation: 1) a qualitative evaluation of carcinogenicity estimating the likelihood that a material might be a human carcinogen, and 2) a quantitative evaluation. In his presentation and subsequent discussions, we were told that the Agency is contemplating, or at least evaluating, the possibility of adopting the IARC criteria and possibly treating genotoxic and nongenotoxic materials somewhat differently. This was not entirely clear, however. The Agency currently uses a multistage model, the lower range of which amounts to a linear extrapolation on the upper 95% confidence interval. Its use has a number of justifications, e.g., when an agent is clearly a genotoxic carcinogen or as a default case (when its carcinogenicity is not known). There was some discussion regarding the possibility of differentiating genotoxic materials from those that do not have a direct interaction with DNA. These might be treated differently as epigenetic carcinogens for which the mechanism of carcinogenicity is less understood but might involve a NOEL or a different type of extrapolation than that of the regular Global 79 model extrapolation. Some problems were pointed out in CAGs present averaging of dose by time weighing. The CAG indicated that they are looking for possibly better models of accomplishing dose averaging, since the present method, in some ways, appears to violate some of the basic concepts of the multistage model. CAG also indicated that human data are fitted linearly on a case-by-case basis.

When it came to the discussion of chemical mixtures for which carcinogenicity had been studied, we essentially had only one example of a complex mixture, i.e., diesel exhaust. For this Dr. Albert indicated the possibility of using a comparative potency type of approach, since it was not possible to administer a sufficiently high dose of diesel exhaust to produce cancer without having other toxic adverse effects due to noncarcinogenic exhaust constituents. An exposure assessment could not be clearly performed. Apparently it was felt that an exposure assessment was necessary because of the finding that the extracts of diesel exhaust were positive in skin painting and were positive in a number of mutagenicity assays. A fairly lively discussion then ensued regarding interactions of carcinogens and noncarcinogens, and the questions involving direct versus indirect pathways in carcinogenicity.

Most of the second day of the meeting was devoted to a discussion of mixtures per se. Initially, the discussion involved the rather simple approach of the summation of fractional doses and effects as currently used by ACGIH. This is somewhat similar to Dr. Crump's third proposed approach. It is not entirely clear as to how Dr. Crump's numerical treatment differs significantly from that of the ACGIH approach. It appears to be essentially the same. In the case of the ACGIH approach, it is used as an indication that a TLV, accumulated from a number of different toxicants that are related, has been exceeded. This has been used apparently in cases of mixtures by the Agency to compare systemic toxicity from exposure to a number of unrelated chemicals.

In an extensive discussion of exposure assessment, the subject of target populations was extended to include subpopulations at greater risk. The degree of susceptibility was brought up with relation to developmental changes, genetic differences, nutritional deficits, existing disease, behavioral effects, and possibly concomitant or previous exposures. It was questioned whether indeed a 10-fold safety factor, as is currently the use in conventional methodology, would be adequate to protect this population.

It was pointed out that if one considers such groups as pregnant women, children from 1-4 years of age, or people with lung, heart or liver disease as being hypersensitive groups rather than being just a portion of the general dose-response curve, then these groups could separately represent a sizable proportion of the population. These groups might need to be treated separately and considered as responding through separate mechanisms. An initial scheme was suggested as to how the presence of hypersensitive individuals in the population might be incorporated into site-specific risk assessments. It was also pointed out that a few of the hypersensitivities might contribute a sizable variation, extending close to two orders of magnitude. However, this appeared to be relatively small considering other variables. One of the difficulties in utilizing, for instance, the TLVs has been that they are based on young, healthy white males, and it may be possible that quite a few of the people who live around a particular release site of many chemicals may exhibit significantly greater sensitivity than the base population from which TLVs were originally derived. Dr. Piscator indicated an interesting way in which statistics, just by getting the right combinations of circumstances at the extremes of various distributions, could give us apparently hypersensitive populations. Without having to invoke the presence of genetic predisposition, it would be possible to have a small fraction of the population affected and to be definitely further out than a 10-fold factor might include. Some of the major questions were whether some of the hypersensitivities were differences in kind as compared with just degree, and that possibly some hypersensitive individuals might respond entirely differently than the population contributing to the main dose-response curve.

The last subject discussed was the one of biological bases for toxicant interactions and mathematical interactions. Dr. Durkin discussed some biological bases on which chemicals might interact, as well as simple chemical interactions that might then result in a new chemical, this new chemical then producing an effect. Following this he discussed a series of mathematic approaches to try to help us uncover the quantitative relationship that might develop in such interactions. It was pointed out that experimental designs for such particular studies were extremely complex, required very large numbers of animals, and were not very likely to be done. A number of additional and unusual types of interactions were discussed as well as some generalities. The question of absorption of divalent cations as influenced by calcium, phosphate, vitamin K, and iron was discussed. The mathematic modeling that needs to be done on multichemical interactions is quite complicated and was advanced as being in the most average default kind of condition. The modeling must work with available data and should be consistent with what we currently use for the single chemical approach. On that particular basis, Dr. Crump has developed a series of additive types of mathematical treatments for the development of ADIs, or virtually safe doses. There was some question as to whether additivity really occurs at low doses. This was answered to some degree for carcinogens, where at least for several examples it does appear to occur. To what extent this occurs with systemic toxicants is still unknown. A suggestion was made that, in light of the great complexities involved, studies must be conducted to better predict possible antagonism or potentiation involved in chemical interactions, and actual testing of various dump effusia should be performed. It was pointed out that this would probably present significant technical difficulties, and that for the time being one would probably have to rely on modeling data, incorporating those phenomena that may be readily explained.

CONCLUDING COMMENTS

DR. THOMAS CLARKSON

Due to deficiencies in mathematical modeling, it would be useful to compile a listing of chemicals known or expected to be in dump sites that might exhibit different types of interactions -- dose-addition, response-addition, potentiating or synergistic action.

The statistical model now developed to predict the toxicity of individual chemicals should be developed to predict the interaction of mixtures.

For acute effects, a mechanism should be established to deal with a data base and communication system to inform Poison Control Centers.

DR. ROBERT NEAL

In my opinion, we need to spend more time on understanding the biological mechanisms of toxicity of chemicals and the validity of our animal models for estimating risk in man. Entirely too much time is currently being spent on trying to refine the mathematic models used to predict low-dose effects in man. Dr. Skalsky pointed out that toxicologists should take more advantage of the drug testing data to validate our animal models. I strongly support this proposal. This is the only substantial body of data where we have dose-response information in both experimental animals and man exposed to the same compound. This is a valuable resource and should be explored more fully for the purpose of validating our animal models for predicting chemical risk in man.

DR. KENNETH CRUMP

It will be more difficult to develop generic methodology for systemic toxicants than for carcinogens because of the much greater diversity of experimental protocols and methods for reporting data in the case of systemic toxicants. EPA should attempt to foster more uniformity in testing protocols and data reporting.

DR. REVA RUBENSTEIN

There was an unspoken assumption, which I found troublesome; that it is possible to unravel the effects of multiple exposure given the same physiologic endpoints. There are such a large number of single chemical exposures for which there is not yet an established risk level, that it is questionable whether we should (or can) proceed to assessment of multiple chemicals. I suggest that any future workshops focus on ways to quantitatively evaluate the basic assumptions.

In general, the participants spent most of the discussion time restating basic assumptions in toxicology. Many, if not all, of these assumptions are in place because one cannot either measure phenomena more accurately, or frame the appropriate questions to sufficiently restructure the toxicology paradigm. Nevertheless, it is important for the regulatory agencies to understand the absolute limitations of the science, i.e., the areas where uncertainty will always remain.

DR. JULIAN ANDELMAN

The prepared document and the discussion at the meeting did not sufficiently distinguish between criteria being considered for protective vs. predictive purposes, as discussed at previous meetings. Thus, for example, there was a considerable discussion of ADI, which is clearly not appropriate in assessing risk.

Also, the concepts of safety and uncertainty factors should be carefully distinguished. It is clear that a safety factor should not be used in assessing risk.

DR. SHELDON MURPHY

I felt the conference was worthwhile and that it was an effective mechanism for exchange of ideas and positions on the conference topic. I was somewhat disappointed, however, that so much time was dedicated to reviewing principles of the toxicological approaches to risk assessment. It seems to me that essentially all the topics discussed on the first day are standard to all chemical risk assessments and have been discussed and debated often and at length for individual chemical exposures. Although our current methods of risk assessments are imperfect, it seems to me that, if we can't make decisions as to what needs to be done and how to apply scientific principles to assessing the hazards of exposure to individual chemicals, we will never be able to assess the health risks of multichemical exposures.

DR. IAN NISBET

Although the comments on the first day were focused on the complexity of toxicological phenomena, and many exceptions to simple generalizations were pointed out, I do not think that ECAO's current procedures were called seriously into question. There seems to be general support for the basic procedures, provided that the possibility of exceptions is borne in mind.

DR. HERBERT CORNISH

It might have been useful if we had some introductory information on specific chemicals at dump sites. I believe it was pointed out that trichloroethylene occurred in 40% of the sites. Similar data on other commonly occurring chemicals might have helped to focus the discussion.

It is also evident that considerable variability will occur in assessing hazards depending on the nature and concentrations of the toxicants at the site.

There is obviously a need for further studies on the effect of multiple exposures in animals, to provide data needed to assess human risk at dump sites.

DR. MAGNUS PISCATOR

A large number of interesting estimates and formulas were presented. However, there was an obvious lack of hard data.

MR. WILLIAM GULLEDGE

Development of water quality criteria and subsequent water quality standards should not be solely based on quantitative risk assessment. Other factors must be considered in developing compound specific criteria on a national level. Policy issues are factors that must be addressed and can include feasibility of enforcing national standards, cost-effectiveness of complexing with proposed regulations, and political climate for implementing a given regulation. Other technical factors should also be included in the decisionmaking process, and these may include effect of existing waste treatment technology in achieving water quality criteria and alternative regulatory options.

DR. ROLF HARTUNG

There appear to be uneven requirements for the amount of evidence that must be presented before various criteria are set or before various phenomena that are part of the evaluation of the evidence for setting criteria are accepted. It is clear that criteria must be developed in the face of uncertainty, i.e., before all the required evidence is in. Similarly, decisions on species differences, epigenetic vs. genotoxic causation, the use of negative data, may have to proceed before all of the data are in, but when the data present a coherent picture.